

BIOLOGICAL CONTROL OF CITRUS LEAFMINER,
PHYLLOCNISTIS CITRELLA (LEPIDOPTERA: GRACILLARIIDAE)
IN SOUTHWEST FLORIDA

By

MARK ALLEN POMERINKE

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I dedicate my dissertation to my wife, Tracy, and my parents, Dave and Betty Pomerinke. Without their encouragement and support this study would not have been completed.

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Abstract of Dissertation Presented to the Graduate School
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Mark Allen Pomerinke

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Chairperson: Dr. Philip A. Stansly
Major Department: Entomology and Nematology

Citrus leafminer (CLM) (*Phyllocnistis citrella* Stainton) was first detected in Florida in May 1993. In May 1994, the parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae) was introduced into southwest Florida as a classical biological control agent for control of CLM.

A. citricola was released at 52 sites in southwest Florida from May 1994 to September 1995. Four release sites were monitored monthly from 1994 to 1996 to confirm establishment. Dispersal was documented by surveying surrounding citrus orchards around these four sites for the presence of *A. citricola*. Mortality factors of CLM were determined by examining cohorts placed at three different orchards in 1996

and 1997. Cohorts were observed daily, and the developmental stage of each CLM determined. Dead CLM were classified as having died from parasitism, predation or abiotic factors. Survivorship curves were developed to better understand which form of mortality was prevalent and when. In 1997, the effect of grove height on mortality factors of CLM was examined. One orchard of short, medium and tall height trees divided into 3 blocks each was used. Four potted plants, two caged and two uncaged, were placed in each orchard monthly. Number of dead, along with cause of death, and surviving CLM were cataloged. Sites were compared to determine if tree height influenced CLM survivorship or mortality factors.

Establishment was confirmed in spring, 1995. Parasitism from *A. citricola* increased from 2% in May 1994 to 86% in October 1995, while parasitism by indigenous parasitoids fell from 18% to 2% during the same period. *A. citricola* dispersed in all directions with a maximum recorded dispersal distance of 48 km. Five parasitoid species were collected during the study, the introduced parasitoid, *A. citricola*, and four indigenous parasitoids. *A. citricola* was the most abundant parasitoid collected in 1996 and 1997. *Pnigalio minio* (Hymenoptera: Eulophidae) was the most abundant indigenous parasitoid collected. Predation had the greatest impact on CLM survival. Ants were the most prevalent predator observed during the study. Undefined mortality ("heat stress") occurred in first and second instars and was thought to be the result of high temperatures. Survivorship curves showed that *A. citricola*'s greatest contribution to CLM mortality occurred in October and November. CLM survival was higher in short trees, while *A.*

citricola recovery was greatest in tall trees. Tree height did not influence recoveries of indigenous parasitoids or predators.

CHAPTER 1 INTRODUCTION

Citrus spp. (family Rutaceae) were probably introduced into Florida by Spaniards at St. Augustine in 1565 (Jackson 1991). Citrus plantings originally were limited to the east coast, and it was not until Florida was ceded to England in 1763 that commerce in oranges increased. According to Jackson (1991) the first big expansion in citrus production occurred in the 1870s as growers realized the size of the potential citrus market for Florida. Since 1894 freezes have caused the citrus industry to move south. Today, southwest Florida has approximately 179,093 acres of citrus, 20% of the state's total acreage (Commercial Citrus Inventory 1996).

The first damaging citrus pest to arrive in Florida was the long scale, *Insulaspis (Lepidosaphes) gloverii* (Pack.) in 1838, and from 1840 to 1870, it caused serious decline in citrus production. Since then a series of pests invaded Florida. The cottony-cushion scale, *Icerya purchasi* Mask., was introduced into Florida in the 1800s. Damage to citrus trees from cottony-cushion scale was finally brought under control by introduction of the vedalia ladybeetle, *Rodolia cardinalis* (Muls.) (Browning 1994). Prior to 1960 the most damaging insect pest of citrus was the purple scale *Lepidosaphes beckii* (Newman). In 1958 a parasitic wasp *Aphytis lepidosaphes* (Compere), was discovered near Ft. Pierce, Florida. Today, the purple scale is under excellent biological control, and is no longer

considered a serious pest (Knapp 1991). The story of Florida red scale *Chrysomphalus aonidum* (L.) is similar to that of purple scale, a serious pest of citrus until the 1960s, when the parasitic wasp *Aphytis holoxanthus* (DeBach) was introduced for biological control. Florida red scale is now considered an infrequent pest (Knapp 1991). Other pests of citrus included Caribbean black scale, *Saissetia neglecta* (DeLotta), the green citrus aphid, *Aphis citricola* (Van der Goot), black citrus aphid, *Toxoptera aurantii* (Fons.), citrus whitefly, *Dialeurodes citri* (Ashmead), and citrus blackfly, *Aleurocanthus woglumi* (Ashby). Most of these pests cause little damage to citrus due to introduction of natural enemies in classical biological control programs. Within the last five years, three more pests of citrus have been found in Florida. They include the recently arrived citrus psylla, *Diaphorina citri* Kuwayama, the brown citrus aphid, *Toxoptera citricida* (Kirk.), and the citrus leafminer, *Phyllocnistis citrella* Stainton. It was the discovery of citrus leafminer in Florida that initiated this study.

The citrus leafminer arrived in Florida in May 1993 (Heppner 1993). It quickly spread across the state and within six months was found in all citrus-growing regions. Three steps were taken to reduce damage from citrus leafminer. The first was an economic assessment of the damage. The second was a combination of cultural and chemical controls for short term control and classical biological control would be attempted as an economical and environmentally-sound long term solution (Knapp et al. 1995). At the time it was also concluded that relying solely on chemical control would be ineffective, costly and could result in disruption of existing integrated pest management programs (Knapp et al. 1995). It was decided that an introduced natural enemy would be

the best approach to control citrus leafminer. In 1994, Dr. Marjorie Hoy imported the parasitoid *Ageniaspis citricola* Logvinoskaya, from Australia (Hoy and Nguyen 1994).

The objectives of the following research were: 1) release and evaluate the establishment and dispersal of *A. citricola* in southwest Florida; 2) determine mortality factors of *P. citrella*, identify species of indigenous natural enemies and the time of season that they are prevalent; 3) determine the role of orchard location and tree height in limiting mortality from natural enemies and evaluate if indigenous parasitoids interfere with *A. citricola*.

CHAPTER 2
RELEASE AND ESTABLISHMENT OF *AGENIASPIS CITRICOLA*
(HYMENOPTERA: ENCYRTIDAE)

Introduction

Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was detected in May 1993 infesting Persian limes in Dade County, Florida (Heppner 1993). The moth spread quickly across Florida and within several months was found in all citrus growing regions (Knapp et al. 1993). It has since been detected throughout the Caribbean, Central America (Heppner 1995), South America, and most Mediterranean countries (Knapp et al. 1993, Hoy and Nguyen 1997). Citrus leafminer had previously been reported as a major pest of new flush in southeast Asia, Australia, Japan, Taiwan, and South Africa (Heppner 1995). Larvae feed by mining the epidermal layer of leaves (Sonhi and Verma 1966), stems, and occasionally fruit.

Evidence from Australia, India, Japan, and Israel indicates that citrus leafminer can be managed by biological control from indigenous and introduced parasitoids (Peña et al. 1996, Batra and Sandhu 1981, Ishii 1953, Argov and Rossler 1996). Surveys in 1993 and 1994 revealed that the leafminer was attacked by a complex of indigenous parasitoids in the families Eulophidae, Encyrtidae, Elasmidae, Furiptomidae and Pteromalidae (LaSalle and Schauff 1996). Peña et al. (1996) found that generalist indigenous parasitoids

provided low to moderate levels of *P. citrella* parasitism in lime orchards in southeast Florida. They suggest that reduction in effectiveness of indigenous parasitoids may be the result of pesticide applications for the control of citrus pests. Given the apparent low levels of control by indigenous parasitoids and difficulty in achieving good chemical control due to the biology of citrus leafminer (Knapp et al. 1995) the importation and release of additional biological control agents was deemed necessary. Citrus leafminer had been detected in Australia in 1912 (Beattie 1993), and the gregarious parasitoid *Ageniaspis citricola* Logvinovskaya was introduced there in 1989 from southeast Asia (Neale et al. 1995). *Ageniaspis* was imported into Florida from Australia with permission from that government in spring 1994. Federal and Florida state permits to release *A. citricola* from quarantine were granted for adults field-collected as pupae in May of that year (Hoy and Nguyen 1994). *A. citricola* is an internal koinobiont parasitoid of citrus leafminer. Eggs are laid into eggs or first instar leafminer and parasitoids pupate inside the host pre-pupa (Edwards and Hoy 1998).

Materials and Methods

Parasitoids were received from Gainesville rearing facilities in plastic 50 ml vials containing an average of 100 adults. Wasps were provided with strips of filter paper moistened with pure honey and packed in styrofoam boxes (21 x 21 x 31 cm) cooled with EverCold® gel refrigerant or Freez Pak Icicle®. Shipments were made by overnight courier and arrived midmorning the following day.

Release sites for *A. citricola* were chosen on the basis of a questionnaire which queried growers in the five county area on location, block size, variety, leafminer status, management practices and their willingness to suspend all pesticide applications for one year post-release. In 1994, 1995 and 1996, 13, 39 and 2 releases were made in 10, 25 and 2 orchards, respectively (Figure 2-1). Releases were generally made an hour or so before dusk, in trees with ample young foliage on which eggs and young citrus leafminer were present. Vials were opened and wasps allowed to crawl out onto young leaves introduced into the neck of the vial to facilitate transfer. Wasps were often observed to immediately initiate searching behavior accompanied by antennation, and to pause over leafminer eggs or small larvae, apparently to oviposit.

Sampling for evidence of leafminer pupae parasitized by *A. citricola* was conducted at least once, two weeks after each release in 1994 to confirm presence of a first generation. No attempt was made to confirm establishment at 1995 and 1996 releases. This was due to the close proximity of previous release sites which would have made it difficult to distinguish between establishment and immigration.

Establishment and Apparent Parasitism.

Four 1994 release sites near Immokalee: one in Lee (Youngquest orchard; Figure 2-1, #4), two in Collier (Foundation; Figure 2-1, #2 and Corkscrew orchards; Figure 2-1, #1) and one in Hendry (A. Duda & Sons orchard; Figure 2-1, #24) counties were monitored bi-weekly in 1994 and monthly in 1995 and 1996. An additional location (Rosbough orchard, 45.7 meters west of Corkscrew orchard; Figure 2-1, #3) in Collier

county was added in late 1994 after *A. citricola* was found to have dispersed into that orchard. All orchards were sampled to confirm establishment and overwintering.

Sampling of *A. citricola* was conducted from time of release/discovery at monitored release blocks through late October when citrus normally becomes dormant (Jackson 1991) and reinitiated in May 1995 and 1996. Samples were obtained by randomly examining 100 intact pupal chambers of citrus leafminer or the number encountered in 30 minutes. Pupal chambers were sampled since *A. citricola* and indigenous parasitoids had pupated by the time this developmental stage was reached. The "sausage link" pupae of *A. citricola*, within the host pre-pupa were easily distinguished from pupae of leafminers or indigenous parasitoids (Figure 2-2). The estimate of "apparent" parasitism provided a convenient measure for comparing parasitoid activity among orchards (Southwood 1984).

Dispersal

Dispersal was documented by surveying citrus orchards for the presence of *A. citricola* along transects away from release sites between July and October, 1995. Intact citrus leafminer pupal chambers were examined at all encountered orchards for 30 minutes or until *A. citricola* was discovered, whichever came first. Transects were followed until no more orchards or another release site were encountered. Dispersal was examined from six sites, with a total of 23 non-release sites being sampled. Transects ranged from 1.6 to 30 miles (2.6 to 48.3 km).

Orchard Structure

Release orchards were categorized as either single or multiple aged. Multiple aged orchards contained blocks of trees consisting of the original planting, usually 15 years or older, along with various aged resets, 2 to 5 years old. Since all multiple aged orchards have resets of varying numbers and ages, a orchard was considered multiple age when at least 50% of the orchard was resets. A orchard with 60% or more of the trees being uniform in size and age was considered single age.

Weather

Weather stations were monitored at two sites; Corkscrew orchard and at the University of Florida's Southwest Florida Research and Education Center near Immokalee, Florida. Temperature and humidity (HMP35C probe), wind direction (024A probe) and speed (014B probe) were continuously recorded using a CR10 weather station (Campbell Scientific, Inc. Logan Utah 84321) at both sites in 1995 and 1996.

Results

Weather

Climatic conditions for 1995 and 1996 at the research center and 1996 at Corkscrew orchard are presented in Table 2-1. Temperatures at the research center ranged from highs of 36.1° C and 35.5° C in August 1995 and July 1996, to lows of -1.6° C and -2.7° C in February 1995 and 1996, respectively. Highs at Corkscrew orchard

reached 36.2° C in September, and dropped to -2.5° C in February. On February 4, 17, and 18, 1996, three freezes with temperatures recorded at -2.4, -1.9, and -0.9° C, respectively, at Corkscrew orchard, and -2.7, -2.2, and 0° C, respectively, at SWFREC. An additional freeze was recorded at the research center on January 9 with temperatures dropping to -1.1° C. No freeze lasted more than 9 hours.

Average relative humidity at the research center ranged from 87% in April to 73.1% in November in 1995 and 85% to 79.9% in April and March, respectively, in 1996 (Table 2-1). Average humidity at Corkscrew orchard ranged from 79.5% in March to 89.7% in August, 1996.

Establishment

Wasps were often observed to immediately initiate searching movements after release accompanied by antennation. They appeared to pause over leafminer eggs or small larvae, perhaps to oviposit. Recoveries were made at five release sites in two counties during the May 1995 survey (Table 2-2). Recoveries ranged from 10 of 25 pupal chambers yielding 29 *A. citricola* pupae to 2 of 25 yielding 6 *A. citricola* pupae. A mean 2.3 (SE=0.13, N=35) *A. citricola* pupae per pupal chamber was found in 1995.

During the May 1996 survey, *A. citricola* was recovered at 14 of 28 release sites in three counties (Table 2-2). Four recoveries were made at orchards where *A. citricola* had been released in 1994 and recovered in 1995; the remaining 10 recoveries were made at 1995 release sites. Recoveries ranged from 41 of 100 pupal chambers yielding 94 *A. citricola* pupae to 1 of 100 yielding 2 *A. citricola* pupae. Again, a mean of 2.3 (SE=.06, N=145) *A. citricola* pupae were recovered from parasitized citrus leafminer pupae.

Apparent Parasitism

A detectable reproducing population of *A. citricola* was observed at Corkscrew orchard two weeks after its initial release in 1994. Incidence of pupae parasitized by *A. citricola* increased to 50% within three weeks and remained above 10% through 12 October 1994, climbing to 83% by June 1995 (Figure 2-3A). In contrast, parasitism by native parasitoids, in the genera *Pnigalio* and *Horismenus*, peaked at 30% four weeks following the release of *Ageniaspis*, and declined to 8% by June 1995.

Incidence of *A. citricola* at the Youngquest orchard was documented on 30 May 1995, at 10%, ultimately peaking at 90% by October 1995. In 1996, parasitism was 20% on 20 May, peaked at 51% in June and fell to 41% by September. Incidence of *Pnigalio* and *Horismenus* fluctuated between 1% and 19% during the same time period, peaking in August 1995 at 21% (Figure 2-3B).

A. citricola was recovered on 12 October 1994, with 1% of citrus leafminer of pupae parasitized in the Foundation orchard, and again on 31 May 1995 with a 2% parasitism rate and increased to 51% by mid October. In 1996, *A. citricola* was recovered on 23 July with an incidence of 9%, and increased to 50% by mid September. Parasitism by *Pnigalio* and *Horismenus* fluctuated between 3% and 8% during the same time period, with a maximum of 16% observed on 9 August 1995 (Figure 2-3C).

A. citricola was first recovered at the Duda orchard on 28 November 1994 with an incidence of 1% which increased to 15% on 19 June 1995 and peaked at 86% on 19 October 1995. Parasitism from *Pnigalio* and *Horismenus* fluctuated between 1% and

40% during the same period with maximum parasitism observed on 28 November 1994 at 40% (Figure 2-3D).

A. citricola was discovered on 17 August 1994 at Rosbough orchard, three months following their initial release at the adjacent Corkscrew orchard. This was the first documented example of *A. citricola* dispersal from orchard to orchard in southwest Florida. Parasitism of citrus leafminer pupae by *A. citricola* was 7% on 17 August and peaked at 26% on 6 October 1994. The incidence of *A. citricola* increased over the 1995 season to a peak of 76% by October. High levels of parasitism were again recorded in 1996, reaching 85%. Parasitism from *Pnigalio* and *Horismenus* never exceeded 7% and these species were almost undetectable in 1996 (Figure 2-3E).

Orchard Structure

A. citricola was detected during the 1995 survey in 5 of the 7 multiple-aged release blocks but in none of the 4 single-aged release blocks (Table 2-2). The parasitoid was recovered at 7 of the 11 multiple-aged blocks and 8 of the 17 single-aged release blocks during the May 1996 survey (Table 2-2).

Nine orchards where parasite establishment was not initially documented received more than one release between 1994 and 1996, four were multiple-aged, the remaining six single-aged. Establishment of *A. citricola* occurred in both types of orchard with the probability of successful establishment higher in multiple-aged orchards (70%) than in single-aged orchards (47%).

Dispersal

Dispersal of *A. citricola* was documented between July and October 1995. Maximum dispersal was documented on 25 October from Green Horizon orchard in Collier County to Stoney's citrus orchards, 16.1 miles (25.9 km) to the southwest (Figure 2-4). In July 1996, *A. citricola* was found on Marco Island, 30 miles (48.2 km) south from Green Horizon Orchard.

Discussion

A. citricola appeared to establish more quickly in multiple-aged blocks compared to single-aged blocks. Parasitoid survival may have been favored in multiple-aged blocks by the shelter and microclimate provided by large trees and by the continuous source of host material provided by rapidly growing small trees. In comparison, blocks of uniform age and size are characterized by synchronous flush, and a less stable microclimate that appeared to slow establishment of *A. citricola*. Number of releases required before establishment was documented appeared to be an indicator of the importance of orchard structure. Nevertheless, *A. citricola* established uniformly throughout the region by 1996, demonstrating that overall, conditions are favorable for this species.

These conditions include a long growing season with hot, humid summers and mild winters. Edwards and Hoy (1998) found that survivorship of *A. citricola* was best at humidities between 80% and 95%, which was the average for southwest Florida during most of 1995 and 1996 (Table 2-1). Probability of establishment may have been

enhanced by releasing early in the rainy season, May through August, which maximized the interval of warm, humid weather and ideal growing conditions.

Parasitism of citrus leafminer by *A. citricola* steadily increased at most sites following initial release, whereas the proportion of host pupal chambers with indigenous parasitoids decline (Figure 2-3). *A. citricola* develops internally, pupating in the leafminer pupal chamber. In contrast, indigenous parasitoids of citrus leafminer, such as *Pnigalio* spp., are generalists capable of parasitizing many different leafminers (Browning et al. 1996, Peña et al. 1996). Also, delayed internal development leaves hosts containing *A. citricola* open to parasitism from quickly developing indigenous ecto-parasitoids such as *Pnigalio* spp., which are unlikely to distinguish between parasitized and un-parasitized citrus leafminer (personal observation). Yet, in spite of these apparent disadvantages, *A. citricola* came to dominate the niche provided by citrus leafminer.

Lack of an alternate host means *A. citricola* populations must track host populations, themselves dependent on the cyclic patterns of flush, which is largely arrested during the winter (Jackson 1991) when generalist indigenous parasitoids would have the greatest advantage. As a likely consequence *A. citricola* was largely absent during the May 1996 samples, whereas indigenous parasitoids were relatively abundant. However, *A. citricola* numbers later rebounded, apparently at the expense of indigenous parasitoids. Possibly, the superior host-finding ability that comes with host specificity conferred a key advantage in the competition for hosts.

Superior searching ability was illustrated in the dispersal of *A. citricola* to new orchards. In spite of impediments that a small size (approximately 1 mm in length

according to Evans 1995 and Logvinoskaya 1983) should pose to directed flight, *A. citricola* was able to disperse over large distances, rapidly colonizing widely scattered citrus plantings over an extensive area (Figure 2-4). *Ageniaspis* dispersed equivalent distances in the Indian River, Homestead, and Orlando citrus growing areas (Hoy et al. 1995). Dispersal distances in southwest Florida are consistent with documented dispersal in Louisiana (United States), Bahamas, Honduras, and Australia (Hoy and Nguyen 1997). *A. citricola* is not unlike its leafminer host, which dispersed throughout Florida over a single growing season. Given successful reproduction, overwintering and dispersal throughout southwest Florida, I feel confident that *A. citricola* has become a permanent addition to the fauna of the region, and will contribute a significant amount of natural mortality to *P. citrella* populations.

Table 2-1. Maximum, minimum and average temperature (°C) along with average relative humidity (%) at Southwest Florida Research and Education Center, Immokalee, Florida in 1995 and 1996, and Corkscrew orchard, Collier county, in 1996.

Southwest Florida Research and Education Center - 1995												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Maximum	27.7	30.0	31.1	32.2	35.0	35.0	35.0	36.1	34.4	33.3	31.6	28.8
Minimum	1.6	-1.6	0.0	10.5	17.2	16.6	21.1	21.1	21.6	15.5	5.0	-0.5
Average	16.6	17.2	20.3	23.0	26.6	26.6	27.5	28.0	27.7	25.8	19.7	16.9
Avg RH	80.0	86.2	85.8	87.0	84.4	82.9	82.5	83.2	84.7	87.0	78.7	NA
Southwest Florida Research and Education Center - 1996												
Maximum	28.8	30.0	30.5	33.8	32.7	34.4	35.5	34.4	35.0	32.2	31.1	29.4
Minimum	-1.1	-2.7	1.1	7.2	16.6	16.1	20.5	19.4	18.8	11.1	5.5	3.8
Average	16.6	16.4	18.0	21.6	25.5	26.3	28.3	27.7	27.2	24.1	20.7	18.3
Avg RH	80.8	75.7	73.7	74.1	85.0	83.0	80.0	84.2	83.0	84.1	77.6	80.0
Corkscrew - 1996												
Maximum	30.7	31.3	35.0	34.0	35.3	34.6	35.8	35.6	36.2	32.7	32.0	30.7
Minimum	10.6	-2.5	1.1	6.2	15.6	14.7	19.0	18.9	18.0	10.5	10.1	2.3
Average	18.8	16.9	17.8	21.3	24.8	25.6	27.3	26.1	26.2	23.4	19.8	17.5
Avg RH	84.5	79.5	77.8	79.7	84.0	88.0	86.4	89.4	86.7	89.0	83.9	84.6
NA, not available.												

NA, not available.

Table 2-2. Release sites, orchard structure, number of *Ageniaspis citricola* released, and parasitism levels from samples of 25 and 100 *Phyllocnistis citrella* intact pupal chambers during May 1995 and 1996 surveys of release blocks where *A. citricola* was released in 1994 and 1995, respectively.

Orchard Structure ¹	Date	Total ²	<i>Ageniaspis</i>	CLM	Indigenous Parasitoids ³
			1995/1996 ⁴	1995/1996	1995/1996
1- MA	3 May 94	75	6/0	17/98	2/2
2- MA	16 May 94, 12 Apr 95	225	0/0	25/100	0/0
3- MA	10 Aug 94 ⁵	0	10/10	12/89	2/1
4 - MA	26 Aug 94	200	9/2	11/95	5/3
5 - MA	1, 25 May, 22 Jul 94	515	8/10	16/89	1/1
6 - MA	20, 28 Sep 95	401	0	99	1
7 - MA	19 Jul 95	300	2	82	16
8 - MA	25 Oct 94	109	2/10	21/82	2/8
9 - MA	28 Oct, 17 Nov 94; 22 May 95; 26 Jun 96	1289	0/0	25/100	0/0
10 - MA	13 Sep 95	500	35	59	6
11 - MA	7 Jul 95	600	4	80	16
12 - SA	28 Feb 95	285	19	67	14
13 - SA	23 Dec 94; 16 Mar, 20 Jul 95	750	0/0	20/95	5/5
14 - SA	1, 17, 22 Mar, 31 May 95	1125	0	99	1
15 - SA	26 Oct 94, 20 Jul 95	287	0/0	25/97	0/3
16 - SA	25 Oct 94	119	0/0	18/100	7/0

Table 2-2--continued.

Orchard Structure ¹	Date	Total ²	<i>Ageniaspis</i>	CLM	Indigenous Parasitoids ³
			1995/1996 ⁴	1995/1996	1995/1996
17 - SA	25 Apr, 9 Jun, 4 Aug 95	640	41	58	1
18 - SA	17 May 95	100	3	95	2
19 - SA	16 Jun 95	500	0	40	0
20 - SA	21 Jun 95	235	0	40	0
21 - SA	25 Oct 95	136	0/2	25/97	0/1
22 - SA	16 May 95	300	9	87	4
23 - SA	20 Jul, 4 Aug 95	600	1	89	10
24 - SA	15 Aug 95	100	2	98	0
25 - SA	20 Jan, 5, 22, 31 May 95	921	0	94	6
26 - SA	1 Mar 95	150	1	98	1
27 - SA	27 Jun 95	300	0	98	2
28 - SA	28 Jun 95	300	0	99	1

1. Number corresponds to location of each release site shown on figure 2-1. MA = Multiple

aged orchard; SA = Single aged orchard.

2. Total number of *Ageniaspis* released at each individual orchard from 1994 to 1996.

3. Two most abundant species *Pnigalio* spp. and *Horismenus* spp..

4. Single numbers indicate that sampling was only conducted in 1996.

5. Date *Ageniaspis* was discovered at Rosbough orchard in Lee county.

Figure 2-1. Location of each release site from 1994 to 1996. Dark areas represent citrus orchards. Number corresponds to data shown in table 2-2.

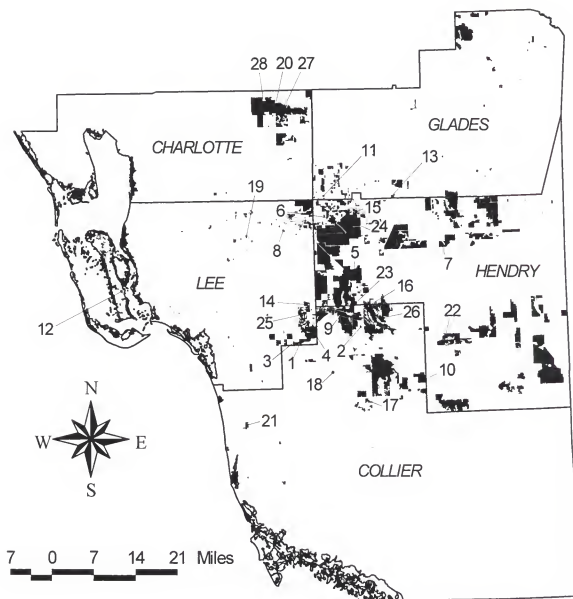
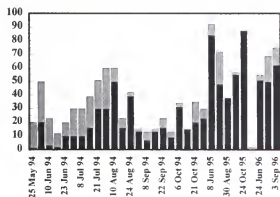


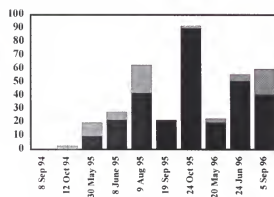


Figure 2-2. Pupae of *Pnigalio minio* (Walker) (Top), *Ageniaspis citricola* Logvinovskaya (Middle), and *Phyllocnistis citrella* Stainton (Bottom).

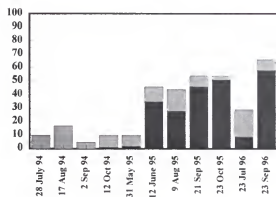
Figure 2-3. Percent apparent parasitism of *P. citrella* pupae by *A. citricola* and indigenous parasitoids at five monitored orchards in southwest Florida. Samples taken weekly from 25 May to 21 October 1994, monthly from 1 May to 24 October 1995 and 23 May to September 1996.



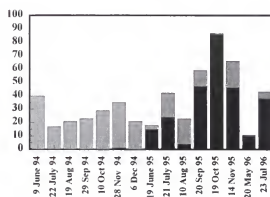
A



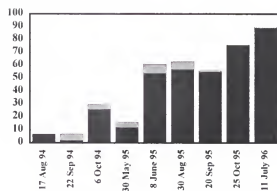
B



C



D

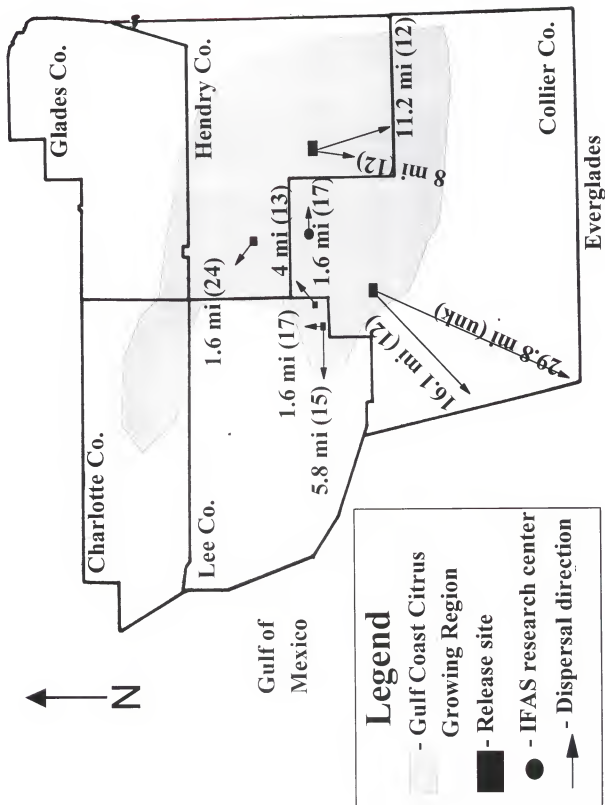


E

■ *Aeniaspis citricola*

■ *Pnigalio & Horismenus sp.*

Figure 2-4. Dispersal of *A. citricola* from six release sites in 1996. Relative direction and distance depicted by arrows. Numbers in parenthesis indicate number of months recovery followed release. All locations sampled once between July and October, 1995. Arrows and map are not to scale.



CHAPTER 3
IDENTIFICATION AND EFFECT OF MORTALITY FACTORS
ON *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE)

Introduction

Evidence from citrus growing regions around the world suggests that *Phyllocnistis citrella* can be effectively managed with biological control (Hoy and Nguyen 1997, Peña et al. 1996). Hymenopterous parasitoids such as *Cirrospilus quadristriatus* (Rao and Ramamani) and *A. citricola* have been introduced into countries where *P. citrella* has established (Hoy and Nguyen 1997). At the same time, indigenous parasitoids and predators have been identified in these citrus growing regions (Chen et al. 1989, Lara-Guerra et al. 1998, Bautista-Martinez 1998, Hoy and Nguyen 1997). For example, Bautista-Martinez et al. (1998) and Lara-Guerra et al. (1998) examined *P. citrella* mortality in the citrus growing regions of Montemorelos, Nuevo Leon, and Cuitlahuac, Veracruz, Mexico, respectively. Lara-Guerra (1998) found parasitism of *P. citrella* by indigenous parasitoids ranged from 32.8% to 100%. Peña et al. (1996) found that in southeast Florida, variable low to moderate levels of *P. citrella* parasitism by indigenous parasitoids occurred in lime orchards throughout the year.

Indigenous predators have been least studied. Chen et al. (1989) examined the feeding behavior of *Chrysopa boninensis* [*Mallada boninensis*] in laboratory studies and

found that this lacewing larva consumed on average 149.1 *P. citrella* larvae in its lifetime. Preliminary cohort studies in 1994 indicated predation was an important mortality factor of *P. citrella* in southwest Florida, with 133 out of 405 (33%) individuals being consumed (Pomerinke unpublished data).

Since *P. citrella*'s arrival in Florida, several indigenous natural enemies have expanded their host range to include *P. citrella*. Indigenous parasitoid species (Chapter 1) and predators in the orders Hymenoptera (ants), Thysanoptera (thrips), Araneae (spiders) and Neuroptera (lacewings) have been documented using *P. citrella* as a food source (Browning and Peña 1995, Hoy and Nguyen 1997). Peña et al. (1996) and Amalin (personal communication) have examined indigenous natural enemies of *P. citrella* in Florida. Their studies focused on either indigenous parasitoids (Peña et al. 1996) or predators (Amalin, personal communication). No study in Florida or elsewhere has yet attempted to evaluate all mortality factors acting on *P. citrella* or documented their effect on leafminer populations.

Native biological control agents could have adverse effects on an introduced parasitoid, interfering with or exploiting host populations, thereby limiting their ability to regulate the prey population (DeBach and Rosen 1991). Evaluation of the nature, abundance and effect of the complex of natural enemies attacking *P. citrella* would aid in understanding *P. citrella* survivorship and any interference that may occur between *A. citricola* and indigenous natural enemies.

Cohorts, individuals of the same age, can be used to determine which life stage of a pest is attacked and when (Southwood 1978). The mortality of marked individuals in

the field is recorded and the data are used to develop a survivorship curve, a graphical description of the survival of a cohort. Mathematical models describing survivorship curves can then be fitted to the data (De Groot and Fleming 1994). Curve shape falls into four categories; types I, II, III and IV. Type I curves are convex and demonstrate that mortality is occurring mostly in later developmental stages. Type II curves are straight lines and show mortality is equally distributed across developmental stages. The type III curve is concave, with slightly higher mortality in early developmental stages. High rates of mortality occurring to early developmental stages are indicated by a strongly-concave type IV curve of exponential form (Southwood 1978).

Types I, II, III and IV survivorship curves might be expected to depend on the predominant guild of natural enemy attacking *P. citrella*. Non-specific predation might act equally over all age classes leading to a type II or III curve. Indigenous parasitoids are also generalists, but their actions are limited to later developmental stages leading to a type I curve. *A. citricola* attacks the egg stage of *P. citrella*, emerging in the pupal stage, causing a type I curve to develop if it is the dominant mortality agent. In addition, curve shape may change temporally, due to differences in climatic conditions, alternate host availability, and changes in prey density. For example, climate and prey density may influence ant foraging (Hölldobler and Wilson 1990). When ants are actually recruiting to leafminer a type II curve might result, whereas when prey densities are low or climatic conditions are less favorable for ants (i.e., spring and fall), the curve may change to a type I, under influence of the parasitoid guild.

This study was initiated to evaluate the relative prevalence of natural mortality factors of *P. citrella* in southwest Florida in 1996 and 1997. Data were used to develop survivorship curves in an effort to understand how the different guilds of natural enemies influence survival of *P. citrella*.

Materials and Methods

1994 Cohorts

Three orchards (Silver Strand, Corkscrew, and Everglades) were chosen for a preliminary cohort study in 1994. Cohorts consisted of eggs oviposited on potted trees by the naturally occurring population of *P. citrella*. *P. citrella* eggs (37) were marked and followed at Silver Strand from 11 to 23 August. On 26 August to 15 September, 54 *P. citrella* eggs were marked and followed at Corkscrew. Two sets of cohorts were followed from 7 to 21 October, one at Corkscrew (107 eggs) and the other at Everglades (207 eggs). Cohorts were examined daily, and the developmental stage of each *P. citrella* determined. Mines and/or dead or missing *P. citrella* were examined to determine cause of mortality. Dead *P. citrella* were categorized as having been killed by *A. citricola*, indigenous parasitoids, predators, or undetermined mortality. Mortality resulting from *A. citricola* was characterized by the presence of multiple pupae in *P. citrella* pupal chambers (Figure 2-1). *P. citrella* killed by indigenous parasitoids was characterized by the presence of a parasitoid larva or pupa associated with the leafminer (Figure 2-1). *P. citrella* dead from predation was characterized by absence of the larva and a small opening

in the mine. Individuals not fitting the above descriptions were recorded as having died from an undetermined cause.

1996 and 1997 Cohorts

Mortality of *P. citrella* cohorts was studied in 1996 to 1997 at three orchards in Collier County, Florida. Two orchards consisted of multiple-aged 'Hamlin' orange, *Citrus sinensis* (L.) Osbeck, trees ranging in age from 6 to 30 years old. The third was a 'Hamlin' orchard of more uniform age consisting of trees 7 years old.

Cohort preparation

Four hundred 'Swingle Citrumelo', *Citrus paradisi* x *Poncirus trifoliata*, trees potted in (15.24 x 40.64 cm) 7.57 liter pots were grown and maintained at the University of Florida, Southwest Florida Research and Education Center (SWFREC) in Immokalee. Every week, 40 trees were trimmed of all their leaves and placed in a 3.65 x 7.32 x 3.65 m mesh screen house (Figure 3-1) covered with 52 x 52 mesh (0.3 mm openings) screen (Lumite, 6525 The Corners Parkway, suite 115 Norcross, GA 30092). When new flush was approximately 3 cm long, trees were placed in small 76.2 x 114.3 x 94 cm wooden cages covered with 52 x 52 agricultural screen (Figure 3-2) for infestation with *P. citrella*.

Adult *P. citrella*, reared from colonies maintained at SWFREC following the methods of Smith and Hoy (1995), were used to infest flush. Pupal chambers of *P. citrella* were trimmed from trees and placed into clear 30.4 x 25.2 cm bags. Bags were then inflated with air and placed under a fluorescent light in the laboratory. After 7 to 10 days, emerging adults were aspirated out of the bags into small plastic vials. Adult moths were fed honey streaked onto tissue paper and allowed to feed overnight. The following

morning adult moths were released into cages containing trimmed trees with small flush. Approximately 100 to 150 adult leafminer were released into each cage. Remaining adult *P. citrella* were placed into separate cages with trees to maintain the colony. A 50/50 sex ratio was assumed. Trees were exposed to adult *P. citrella* for four nights to obtain sufficient eggs for each replication ($\approx 100/\text{cohort}$, total 600/replication). Leaves were then examined for leafminer eggs, and sequentially numbered using a ultra fine point permanent black marker. Five to 30 eggs were marked on each tree, averaging 20 marked eggs/tree. Although it was not possible to know the exact age of each egg, age was assumed to be on average 2 days old when marked, and all eggs hatched within one day of each other.

During 1996 and 1997, cohorts were placed at Jackson orchard instead of Everglades orchard because the latter was often sprayed with pesticides. Two marked cohorts of approximately 105 eggs each were randomly assigned to one of three groves (Silver Strand, Everglades or Corkscrew) at the beginning of each month from March to November, 1996 and April to October, 1997. Placement of pots at each site was not synchronized to flushing patterns of established citrus in southwest Florida. Each pot was placed into a shallow hole for support. One of each pair of cohorts designated as "ant-free" was randomly chosen to be protected from ants by a barrier, while ants were encouraged to forage on the second "ant" cohort. "Ant-free" trees had a one inch wide strip of Tanglefoot® insect trap coating (The Tanglefoot Company, Grand Rapids, Michigan 49504) painted on the pots 2-3 inches from the top. If ant activity was noted during daily observations, a new strip of Tanglefoot® was painted on the pot and all

visible ants removed. "Ant" trees had a string tied to the corner of the pot and attached to an adjacent citrus tree. Honey was placed on the string to encourage ant foraging.

Mortality types

As in 1994, cohorts were examined daily, and the developmental stage of each *P. citrella* determined. Mines and/or cadavers of dead or missing *P. citrella* were examined to determine cause of mortality. Mortality categories were expanded in 1996 and 1997. Dead *P. citrella* were categorized as having been killed by *A. citricola*, indigenous parasitoids or predators, weather or competition. Host feeding by parasitoids was determined by the presence of a small black spot on the dorsal side of an intact, dead or immobilized larvae in the mine.

Predation was subdivided into mortality resulting from ants, spiders or spiderlings, and undefined predators. Ants were observed to remove whole *P. citrella* larvae cleanly from mines, leaving only a small hole in the leaf cuticle over the mine or through the leaf at the back of the pupal chamber. Spiders took *P. citrella* close to shelters by puncturing the leaf cuticle to penetrate the mine, leaving the larval cuticle in a fluid-filled mine. Mortality not fitting the above descriptions, but apparently resulting from predation, was recorded as undetermined predation. Undetermined predation took several forms: 1) larvae missing from mine with severe mine damage, 2) half eaten larva in mine, and 3) larva in mine and not intact, with liquid in the mine.

P. citrella removed from mines as a result of leaves rubbing against other plant parts were recorded as having been killed by wind. This was typified by the disrupted mine and small scratches on leaves.

Intraspecific interference also was recorded during the study. This occurred when two or more individuals fed on the same leaf. Typically one larva would feed in the path of the other, stranding the second larva in the mine, ultimately causing its death. Finally, any dead individuals not fitting the above descriptions were recorded as having died from an undetermined cause and, most often appeared desiccated. *P. citrella* natural enemies collected during the study were identified by G. Evans and M. Deyrup (Hymenoptera), and J.P. Michaud (Neuroptera).

Following pupation, the numbers of emerging adult leafminers, *A. citricola*, and native parasitoid pupae were counted. Parasitoid pupae were returned to the laboratory, placed in individual petri dishes in a desiccator, and allowed to emerge. Adult parasitoids were then identified, sexed, and counted. *A. citricola* adults were sexed following the methods of Evans (1995).

Because different numbers of *P. citrella* were observed monthly, results were log transformed ($\log +1$). One cohort in May and two cohorts in both June and July 1996 were sprayed with pesticides killing most of the developing *P. citrella*, therefore these cohorts were dropped from all analysis. A one-way ANOVA was then performed to determine whether any significant difference occurred among mortality factors in 1996 and 1997 (SAS 1988). Data from 1994 were not analyzed using ANOVA because only 4 cohorts were followed and then only in the last three months of the year. Chi-square (PROC FREQ, SAS 1988) was used to compare the relative importance of mortality types between months and sites, using untransformed data in 1994, 1996 and 1997. Correlation

analysis (PROC REG, SAS 1988) was used to examine whether the number of leafminers killed by ant predation and heat stress was correlated to increasing temperature.

In addition to potted plants, three cohorts consisting of approximately 50 naturally occurring *P. citrella* eggs were marked in August 1996 and 100 eggs each in April and May 1997 on established trees to compare with results obtained from potted plants. Individual cohorts were considered a replication and a one-way ANOVA analysis was performed to determine differences between treatments (SAS 1988). Month of cohort placement along with number of cohorts and the total number of marked *P. citrella* in each month are shown in Table 3-1.

Survivorship curves

Survivorship curves were developed from life-expectancy tables for each cohort by plotting number of live *P. citrella* per day against the number of days each cohort was monitored (Southwood 1978, Slobodkin 1962). Survivorship curves were visually placed into one of four types based on their shape and regression analysis performed. Four regression models were chosen using SigmaPlot® 4.0 (Jandel Scientific 1986) for comparison with survivorship curve types based on the shape of curve each model produced. The Gompertz, 3 parameter sigmoidal model (Equation 3-1) for the type I curve, a linear model (Equation 3-2) for type II, a hyperbolic decay model with 3 parameters (Equation 3-3) for type III, and a single exponential decay model with 2 parameters (Equation 3-4) for type IV (Table 3-1 and Figure 3-3). The model producing the highest r-squared value was considered to best define the curve. All regression analyses were performed using SigmaPlot® 4.0 (Jandel Scientific 1986).

$$\text{Type I: } f = a \cdot \exp(-\exp(-(x-x_0)/b)) \quad (\text{Equation 3-1})$$

$$\text{Type II: } f = y_0 + a \cdot x \quad (\text{Equation 3-2})$$

$$\text{Type III: } f = y_0 + (a \cdot b)/(b + x) \quad (\text{Equation 3-3})$$

$$\text{Type IV: } f = a \cdot \exp(-b \cdot x) \quad (\text{Equation 3-4})$$

Values of the constants in these equations have been inserted in the corresponding graphs in Figure 3-3.

Results

1994 Preliminary Cohort Study

Percentage of *P. citrella* adults recovered was not significantly different among months ($X^2 = 0.159$; $df = 2$, $P > 0.05$) (Figure 3-4). A significant difference in recovery of *P. citrella* adults was observed between Everglades (1%) and Corkscrew (16.5%) orchards in October ($X^2 = 22.67$; $df = 1$; $P < 0.05$). Of the mortality factors recorded in 1994, predation was the most important type in August and September with 43% and 53% of *P. citrella* consumed by predators, respectively (Figure 3-5). Predation was not significantly different between August and October ($X^2 = 3.79$; $df = 2$; $P > 0.05$) (Figure 3-5). No significant difference was observed between sites in percent predation ($X^2 = 2.90$; $df = 2$; $P > 0.05$).

A. citricola was only recovered in September and October from cohorts monitored at Corkscrew, while indigenous parasitoids were recovered from all sites during each

month (Figure 3-7). Recoveries of indigenous parasitoids was not significantly different among months ($X^2 = 3.97$; $df = 2$; $P > 0.05$) or sites ($X^2 = 3.65$; $df = 2$; $P > 0.05$).

Undetermined mortality accounted for 30% of the mortality in August, increasing to 32% by October (Figure 3-5). No significant differences were observed in undetermined mortality among months ($X^2 = 2.98$; $df = 2$; $P > 0.05$) or sites ($X^2 = 0.62$; $df = 2$; $P > 0.05$).

Survivorship curves (lx) were developed from mortality tables in appendix I. The August data are best described by an exponential decay model with 2 parameters (equation 3-4) and therefore conformed most to a type IV survivorship curve indicating heavy mortality in egg and early instar developmental stages (Table 3-4). Remaining curves from September and October were best described by the hyperbolic decay model with 3 parameters (equation 3-4) and thus were best described as type III curves indicating mortality occurred equally in all developmental stages (Table 3-4).

1996 and 1997 Cohorts

Adult Survivorship

P. citrella mortality was not significantly different between ant and ant-free treatments in 1996 and 1997 ($F = 5.29$; $df = 3,64$; $P = 0.1928$). This appears to be due to ants circumventing the Tanglefoot painted on the "ant-free" pots. Therefore, data were combined and, unless otherwise noted, treatments are discussed together.

Percentage of adults recovered (survivorship) from cohorts in 1996 was highest in spring, dropping through the summer into fall. For example, greatest survivorship occurred in April (79.4%), with lowest survivorship in November (18.6%) (Figure 3-4).

However, October cohorts did not follow this trend; more *P. citrella* (56.8%) survived during this month compared to August and November ($X^2 = 227.31$; $df = 2$; $P < 0.001$) (Figure 3-4). A significant difference was observed in the proportion of *P. citrella* adults recovered between sites, with more adults being collected at Silver Strand (30.3%) followed by the Corkscrew (29%) and Jackson (21.3%) orchards ($X^2 = 23.47$; $df = 2$; $P < 0.001$).

In contrast to 1996, *P. citrella* survivorship in 1997 was constant, not exhibiting the increase in spring followed by the steady decline in the fall (Figure 3-4). As shown in figure 3-4, the percentage of *P. citrella* adults fluctuated from 43.6% to 43% from April to October, respectively, with a minimum recovery of 28.1% in May. As in 1996, a significant difference occurred between sites in the proportion of leafminer adults recovered. Silver Strand (13.4%) ($X^2 = 37.93$; $df = 1$; $P < 0.001$) and Corkscrew (16.4%) ($X^2 = 26.97$; $df = 1$; $P < 0.001$) orchards had a significantly lower percentage of leafminer adults recovered compared to the Jackson (25.1%) orchard, but were not significantly different from each other ($X^2 = 3.56$; $df = 1$; $P > 0.05$).

Mortality Type and Occurrence

A total of 4,738 (74.7%) *P. citrella* died of 6,336 marked individuals from cohorts in 1996 and 1997. Mortality in 1996 was typically heaviest from May to November, while mortality in 1997 was above 50% in April and remained high throughout the year (Figure 3-5).

Predator Guild

Predation tended to be lowest in the spring and fall and highest during mid-summer of both years. For example, in 1996 predation in May was 16%, peaking in July at 48.8%, dropping to 5.1% by November. In 1997 predation rates were again low in spring (29.1% in April), peaking in May at 47.4%, and reached a minimum of 17.5% in October (Figure 3-5).

Nine species of Formicidae were collected from potted plants in "ant" and "ant-free" treatments. These included *Brachymyrmex obscurior* Forel, *Solenopsis invicta* Buren, *Dorymyrmex bureni* (Trager), *Hypoponera punctatissima* (Roger), *Pheidole moerens* Wheeler, *Tapinoma melanocephalum* (Fabricius), *Pseudomyrmex gracilis* (Roger), *Crematogaster ashmeadi* Mayr. and *Paratrechina bourbonica* (Forel). Two species were observed feeding on *P. citrella*: *P. gracilis* and *C. ashmeadi*. Species in the genera *Solenopsis*, *Pheidole*, and *Paratrechina*, although not seen feeding on *P. citrella*, are known to feed on insects (Creighton 1950, Deyrup and Trager 1986).

Tanglefoot painted around potted plants failed to keep ants from consuming marked individuals in "ant free" cohorts. Ants, specifically *C. ashmeadi* and *D. bureni*, were observed foraging on and in "ant free" trees. *D. bureni* was collected from underneath pots in 1997, whereas *C. ashmeadi* and *S. invicta* were found in pots kept in screen houses at SWFREC. In an attempt to exclude ants from potted plants, trees were moved away from branches of established trees and additional tanglefoot was painted around pots. However, ants still managed to cause mortality on "ant-free" trees. They probably circumvented the tanglefoot by tunneling through the soil in the pots, a behavior

noted in the screen house. Bottoms of potted plants were not closed because citrus trees require drainage. Due to the need to handle foliage and keep it from become sticky, Tanglefoot was not painted onto tree trunks. In addition, the variable depth each tree had been planted into its pot made it difficult to reach clean areas on some trunks.

Ants were the most abundant predator, accounting for 29.7% of all predation observed (Table 3-2). Overall, the percentage of leafminers killed by ant predation in 1996 increased in spring, peaked in mid-summer and declined slightly in the fall. For example, lowest ant predation occurred in March (47%), highest in July (87%). This trend was not observed in 1997 with predation by ants starting out high in the spring (April), and remaining high until late fall (October) (Figure 3-6). Percentage of leafminer consumed by ants was significantly different between orchards in 1996 and 1997. A significantly larger percentage of leafminer was consumed by ants at Silver Strand (1996 = 22.8%, 1997 = 46.4%) compared to Corkscrew (1996 = 12.5%, 1997 = 20.7%) and Jackson (1996 = 18.3%, 1997 = 32.5%) in both 1996 ($X^2 = 39.84$; $df = 2$; $P < 0.001$) and 1997 ($X^2 = 129.67$; $df = 2$; $P < 0.001$).

In August 1996 and April and May 1997, naturally-occurring cohorts were monitored on established trees at Corkscrew to ascertain whether mortality of *P. citrella* on potted plants was equivalent to mortality on established trees. Thirty-two of 256 *P. citrella* (12.5%) from established tree cohorts were consumed by ants, which was not significantly different from potted cohorts observed at the same site in August 1996 ($X^2 = 1.08$; $df = 1$; $P > 0.05$), but was significantly different in April ($X^2 = 8.22$; $df = 1$; $P < 0.004$) and May ($X^2 = 17.81$; $df = 1$; $P < 0.001$) 1997.

Two species of Chrysopidae, *Chraeochrysa lineaticornis* (Fitch) and *Chrysoperla rufilabris* (Burmeister), were observed searching for food on *P. citrella* infested flush. *C. lineaticornis* immatures accepted *P. citrella* larvae still in the mine when offered them in the laboratory. However, no evidence of predation or activity by lacewings was observed on potted plants.

Spider predation was one of the least significant mortality factor among predators, accounting for 1.5% of all predation (Table 3-2). Few spiders (n=31) were observed on potted plants in both treatments, and therefore were not identified to species. Consumption of first and second instar *P. citrella* larvae by spiders averaged 5 ± 3.91 (mean $\pm$ SD) individuals eaten/month from 1996 to 1997. Spider predation was highest in March 1996 and October 1997 (Figure 3-6).

Mortality caused by unknown predators was the fourth most important form of mortality accounting for 7.6% of observed predation (Table 3-2). In general, more unknown predation was observed in 1996 than 1997 with the greatest amount occurring in October 1997 with 54.3% of *P. citrella* killed by unknown predators (Figure 3-6).

Parasitoid Guild

Similar trends in parasitism were observed in 1996 and 1997 with parasitism starting out low in both years, peaking by late fall. For example, parasitism was 7.5% in March 1996, slowly increasing to 58.7% in November, while in 1997 9.0% of *P. citrella* were parasitized in April, increasing to 36.1% in October (Figure 3-5).

Parasitoids collected during the study included: *A. citricola* (Encyrtidae), *Pnigalio minio* (Walker), *Horismenus sardus* (Walker), *H. fraternus* (Fitch) (all Eulophidae), and

Elasmus tischeriae Howard (Elasmidae) (Table 3-3). *A. citricola* was the most abundant parasitoid collected (parasitized pupal chambers of *P. citrella*), killing a greater percentage of *P. citrella* (15.9%) than all other parasitoids combined (3.8%) (Table 3-2). *P. minio* (3.6%) was the most abundant indigenous parasitoid collected, followed by *Horismenus* sp. (2.3%), and *E. tischeriae* (1.6%) (Table 3-3). Undetermined species and individuals that died during rearing accounted for 10.9% of recovered indigenous parasitoids. Although these parasitoids died in the laboratory during rearing and therefore could not be identified, they were likely the same species as those reported. Parasitism accounted for 18.7% of *P. citrella* cohort mortality observed during the study.

Parasitoid species varied by time during both years of the study (Figure 3-7). For example, *Horismenus* sp. was the only parasitoid collected from a cohort in March 1996, while *P. minio* was not collected until June (Figure 3-7). In addition, *A. citricola* was not collected in a cohort until June, while *E. tischeriae* was collected only in August and October (Figure 3-7). Indigenous parasitoid recoveries remained low, but detectable, throughout 1997 showing the same variation in species over time (Figure 3-7). In contrast to 1996 when *A. citricola* was first collected in June, *A. citricola* was recovered in April during 1997. In addition, at no time in 1997 did *A. citricola* recoveries account for less than 92% of all parasitoids recovered (Figure 3-7).

When *A. citricola* recoveries from cohorts were examined by orchard it was found that in 1996 significantly fewer were recovered at Corkscrew (3.6%) compared to Silver Strand (8.3%) and Jackson (22.5%) ($X^2 = 151.57$; $df = 1$; $P < 0.001$). This trend in recoveries reversed in 1997 with more *A. citricola* being recovered at Corkscrew (19.6%)

compared to Silver Strand (11.0%) ($X^2 = 37.04$; $df = 1$; $P < 0.001$) and Jackson (10.9%) ($X^2 = 42.47$; $df = 1$; $P < 0.001$), while Silver Strand and Jackson were not significantly different ($X^2 = 0.001$; $df = 1$; $P > 0.05$).

Recovery of indigenous parasitoids from cohorts was significantly different between sites in 1996, with more parasitoids being recovered at Jackson (4.2%) followed by Corkscrew (2%) and Silver Strand (9.4%) orchards ($X^2 = 73.97$; $df = 2$; $P < 0.001$). In contrast, no significant difference was observed in recoveries of indigenous parasitoids from cohorts at sites in 1997 with 2.2%, 2.5% and 1.7% of indigenous parasitoids recovered at Corkscrew, Silver Strand, and Jackson orchards, respectively ($X^2 = 1.28$; $df = 2$; $P > 0.05$). Host feeding by indigenous parasitoids was identified in both years, but accounted for only 1.2% of all mortality (Table 3-2).

Additional Factors

Mortality was observed from wind causing leaves to rub together, removing leafminer larvae. Wind was the least significant form of mortality, killing only a small percentage (1.3%) of *P. citrella* larvae (Table 3-2 and Figure 3-8).

Undetermined mortality, believed to be most likely "heat stress", typically occurred to first to second instars, and was easily identified by looking for a dry, brown, flat outline of the larva at the end of a short mine. The highest percentage of "heat stress" occurred during summer months, for example June 1996 and May 1997 (Figure 3-8). Highest levels of "heat stress" occurred in months when high temperatures were recorded at SWFREC and appeared to be correlated with temperature in both years (1996: $r = 0.66$ and 1997: $r = 0.85$). This type of mortality was the second most important following

predation caused by ants, accounting for 17.4% of the total observed mortality (Table 3-2).

A few late instar larvae were unresponsive, intact and in the mine with whitish colored hemolymph or black in coloration. The exact cause of mortality is unknown.

A small number of *P. citrella* (1.5%) died as a result of intraspecific interference. Interference arose when multiple *P. citrella* larva fed on the same leaf. Interference was one of the least significant form of mortality observed (Table 3-2).

Survivorship curves

Survivorship curves (lx) developed from mortality tables (Appendix I) varied from type I to IV (Table 3-4). Survivorship curve (lx) type changed from spring to fall in 1996 and 1997. For example, curve shape was predominantly type II and III from March to August in 1996, indicating that predation and "heat stress" were the main components of mortality. However, by October and November survivorship curves became type I, indicating, like the data shows in figure 3-5, that parasitism, specifically from *A. citricola* (Figure 3-7), became the main component of mortality in these months. The same trend in curve types occurred in 1997. Curves from April to August were predominantly type II and III, due to predation and "heat stress", predominantly becoming type I by October due to parasitism (Table 3-4).

Discussion

The number of surviving *P. citrella* in cohorts declined (Figure 3-4) due to two natural enemy guilds, coupled with an abiotic factor: Initial decline in early spring and summer was from predation, with a continued decline of *P. citrella* populations in late summer and fall primarily due to increasing parasitoid activity, in particular *A. citricola* (Figure 3-7). In addition, “heat stress” appeared to be a significant form of *P. citrella* mortality throughout the study.

Ant predation was responsible for more *P. citrella* mortality than any other type of mortality observed (Table 3-2). This was not unexpected because among invertebrate predators, ants are probably the primary source of leafminer mortality (Hespenheide 1991). For example, Hinkley (1963) attributed an outbreak of two lepidopteran leafminers to the elimination of ant predation after an application of dieldrin to sweet potatoes in Fiji.

Ants, however, were not the primary source of mortality throughout the entire study. The exact reason for reduced activity is not known although foraging temperature preference and prey abundance may have influenced ant predation. Daytime optimal foraging temperature for *S. invicta* in Florida ranges from 22-36° C (Porter and Tschinkel 1987). Average minimum temperatures in southwest Florida did not reach and stay within this range until May 1996 and March 1997, dropping below this in November 1996 and 1997 (Obreza 1998). Optimal temperature ranges for foraging are unknown for the other

ant species collected. However, if ranges roughly correspond to that of *S. invicta*, then reduced foraging would leave more *P. citrella* to be used by other natural enemies.

In addition to foraging temperature, prey abundance may have influenced ant predation. From October to March, trees arrest or reduce growth of new foliage (Jackson 1991). Reduced flushing would decrease prey and honeydew that may initially induce ants to forage on trees. From March to June 1996 and April to June 1997, cohorts had highly concentrated populations of *P. citrella* compared to surrounding trees. In April 1997, 10 potted plants averaged 12.8 ± 4.70 stems/m² and 20 *P. citrella*/tree compared to 10 established orchard trees with 3.7 ± 3.80 stems/m² and less than 1 *P. citrella*/tree. Once ants found the potted trees they fed on the relatively abundant *P. citrella*. In contrast, the same level of mortality was not observed from naturally occurring cohorts on established trees in April and May, when *P. citrella* populations tend to be lowest. In August 1996, the percentage of leafminer mortality from ants was the same on potted plants and established trees. This may indicate that when *P. citrella* populations are high, more *P. citrella* are consumed by these generalists. Given the amount of mortality attributed to ants in cohorts, it appears that Formicidae could be the single most important cause of *P. citrella* mortality from late spring to mid summer in southwest Florida.

Evidence of *P. citrella* predation by Araneae also was recorded from cohorts. Several Araneae were observed on potted plants and probably arrived as spiderlings ballooning on silk (Riechert and Lockley 1984, Greenstone 1982). Upon arrival, spiderlings made small shelters by tying several leaves together with silk. *P. citrella* predation was limited to larvae near shelters. As spiders aged they appeared to lose

interest in *P. citrella*, even when larvae were nearby (personal observation). This same behavior was observed in spiders collected in lime groves around Homestead, Florida (D. Amalin, personal communication). Amalin speculates that older spiders may look for large, active prey. However, on several occasions *P. citrella* larvae were observed to complete development adjacent to shelters built by spiders that had preyed upon marked leafminer larvae.

No cohort mortality was attributed to *Chrysoperla* during the study. Cohort preparation, specifically trimming all foliage from potted trees, effectively discouraged *Chrysoperla* oviposition. Lack of lacewing activity in the cohorts does not mean that they do not contribute to the overall mortality of *P. citrella*. Chrysopidae do feed on Lepidoptera (Metcalf and Metcalf 1993), and have been documented to feed on *P. citrella* (Chen et al. 1989, Knapp et al. 1995).

The single most important parasitoid recovered was *A. citricola*. However, recoveries were not consistent. *A. citricola* was recovered only at Corkscrew in 1994. Lack of recoveries at the other two sites in 1994 was primarily due to the fact that *A. citricola* had just been released and had not yet established at the other sites. Previous studies have shown *A. citricola* difficult to recover in early spring (Hoy and Nguyen 1997). Recoveries during 1996 were no different, following the pattern observed in 1995 after the establishment of *A. citricola* (Chapter 2). As demonstrated by survivorship curves and recoveries in cohorts, the slow build up of *A. citricola* resulted in the greatest effect on *P. citrella* in late summer to early fall (Table 3-4 and Figure 3-7). In contrast, *A. citricola* built up quickly in 1997. Favorable climatic conditions may have favored *A.*

citricola survival (Obreza 1998). Thus, *A. citricola*'s contribution to *P. citrella* mortality in 1997 began earlier due to high overwintering populations. Even with a mild winter in 1996-1997, survivorship curves again show the greatest benefit from *A. citricola* occurred in late summer and fall (Table 3-4 and Figure 3-7).

Indigenous parasitoids were recovered from cohorts in every year. This was expected because parasitism of *P. citrella* by indigenous parasitoids had been recorded prior to the establishment of *A. citricola* (Figure 2-2). In March, June and October 1996, indigenous parasitoids were more prevalent than *A. citricola*, although no more than 22 indigenous parasitoids were recovered at a time. Indigenous parasitoids were never recovered in great abundance in 1997, although they were present and detectable throughout the year (Figure 3-7). The contribution to mortality from indigenous parasitoids appears to have been too slight to have affected *P. citrella* survivorship (Figure 3-7).

Two reasons for the limited effect by indigenous parasitoids may be host specificity and displacement. All indigenous parasitoids recovered are known to be generalists, fortuitously parasitizing rather than specifically seeking out *P. citrella*. *P. citrella* is probably used by indigenous parasitoids because like their other hosts, it is a leafmining lepidopteran. According to Hawkins and Lawton (1987) and Hawkins (1988) leaf-mining insects have a larger parasitoid species load than both less and more concealed hosts. *P. minio* is known to attack leaf-mining or gall-making insects in the orders Lepidoptera, Coleoptera, Diptera, and Hymenoptera (Schauff and LaSalle 1996). *E. tischeriae*, along

with both *Horismenus* spp., attack a wide range of leaf-mining insects and all have also been recorded as facultative hyperparasitoids (Schauff and LaSalle 1996).

Bautista-Martinez et al. (1998) found the percentage of native parasitoids was lowest in August and September compared to the remaining months in 1995 and from January to July 1996 in Cuitlahuac, Veracruz, Mexico. The authors speculate that during these months indigenous parasitoids are using other leafminer species. Temporal variation is not uncommon for leafminer parasitoid guilds and has been reported from *Liriomyza* spp. (Johnson and Hara 1987). Recoveries in cohorts may have been lowest when a preferred host was abundant. *P. minio* has the broadest host range recorded, having been reared from *Brachys* spp. (Coleoptera: Buprestidae), *Exema* spp. (Coleoptera: Chrysomelidae), *Lithocolletis* and *Gracillaria* spp. (Lepidoptera: Gracillariidae), and *Agromyza* and *Liriomyza* spp. (Diptera: Agromyzidae) (Krombein et al. 1979, Yoshimoto 1983, Miller 1970). *E. tischeriae* has been reared from *Tischeria solidaginiforliella* Clem. (Lepidoptera: Tischeriidae), whereas *H. fraternus* has been reared from *Coleophora pruniella* Clem. (Lepidoptera: Coleophoridae), and *Sympiesis* spp. (Hymenoptera: Eulophidae) (Krombein et al. 1979). No host other than *P. citrella* is known for *H. sardus*. However, it seems reasonable to assume that *H. sardus* has a host range similar to that of *H. fraternus*. Unfortunately the host range of indigenous parasitoids in southwest Florida is not well known (Hoy and Nguyen 1997). Lack of information about indigenous parasitoid hosts means host plants are also not well known. However, five species of *Phyllocnistis* occur in Florida along with seven species of *Lithocolletis* and ten species of *Gracillaria* (Kimball 1965), all of which may be hosts of indigenous parasitoids collected

from *P. citrella*. Host plants for these species are known and several of them grow in and around citrus orchards, including wild grape, *Vitis rufotomentosa* Small, and burn weed, *Erechtites hieracifolia* (L.) Raf. (Kimball 1965). Given that all of these *Phyllocnistis* spp. are related, behave similarly, and are found in the immediate area of each other it is not surprising that indigenous parasitoids appear to fortuitously parasitize *P. citrella*.

Limited effects from indigenous parasitoids may also be explained by displacement after the establishment of *A. citricola*. As recovery data show, more indigenous parasitoids were recovered in 1994 and early 1996 (Figure 3-7). By late 1996 and throughout 1997, indigenous parasitoids were only a minor component of the parasitoid guild. This was expected, given that *A. citricola* was released to control *P. citrella*. In contrast, *P. citrella* represents a host expansion for the indigenous parasitoids. As the host-specific parasitoid *A. citricola* became established, it reduced the abundance of *P. citrella*, displacing generalist indigenous parasitoids to more abundant hosts. This displacement is like that observed for parasitoids released to control oriental fruit fly, *Dacus dorsalis* Hendel in Hawaii, citrus blackfly *Aleurocanthus woglumi* Ashby in Florida, and California red scale *Aonidiella aurantia* (Maskell) in California (DeBach and Rosen 1991). In each case, the most efficient parasitoid came to dominate and as shown by recovery data this is *A. citricola* in Southwest Florida (Figure 3-7).

The second most important mortality type in cohorts was "Heat stress" (Figure 3-8). "Heat stress" tended to occur among first and second instars. Larvae were typically found in mines, desiccated and intact with no sign of mine destruction or feeding by natural enemies. Unwin and Corbet (1991) state that the temperature of one leaf can vary

quite markedly from another. The surface of a mine is like a glass window and even moderate exposure to the sun could cause trapped air to become hot, desiccating a young *P. citrella* larva. High temperatures during months with the highest level of "heat stress" averaged 25°, 34°, and 33° C in March, July, and August, 1996, respectively, and 28° and 34° C in April and May, 1997, respectively. Ba-Angood (1977) speculated that similar mortality of *P. citrella* in Sudan was caused by high temperatures, albeit temperatures higher than in Florida.

On a few occasions late instar *P. citrella* were discovered intact in their mine with a milky white or brown coloration. The milky white coloration may have been caused by fungi or bacteria. However, no investigation for pathogens or microsporidia was performed during the study. The black coloration could have been caused by an indigenous parasitoid, *Cirrospilus* spp., which kills the larva, causing the host to decompose quickly, with the contents becoming a black liquid (Knapp et al. 1995). No *Cirrospilus* spp. were collected during the study. It is more likely these individuals died from host feeding by indigenous parasitoids and the puncture mark went undetected.

Intraspecific interference was the second least significant form of *P. citrella* mortality in cohorts. Competition resulted when a *P. citrella* larva fed under an egg or in the path of another larva. The resulting loss of mesophyll effectively strands a developing *P. citrella* in the mine, leaving it unable to forage, ultimately starving to death (Murai 1974). Natural intraspecific interference is most prevalent during periods of light flushing (personal observation). Egg hatching becomes irregular and *P. citrella* that hatch first outcompete surrounding individuals. Mortality from intraspecific interference appears to

have been caused by infesting plants in cages. Due to insufficient oviposition sites, female *P. citrella* oviposited multiple eggs per leaf, simulating a period of limited flush.

Also interesting during the study were the differences observed in adult survivorship and mortality types occurring at different sites. A combination of factors may explain these differences. First, Corkscrew and Jackson orchards appear to be more conducive to *A. citricola* survival, perhaps due to higher humidity. Edwards and Hoy (1998) have shown that low humidity adversely affects the survivorship of *A. citricola*. Higher humidity may be the result of Corkscrew and Jackson orchards having older established trees mixed with smaller resets compared to Silver Strand that had trees of uniform height and age. Cloudsley-Thompson (1962) indicates that orchard architecture can influence microclimate. Second, differences in ground cover may affect indigenous parasitoid host availability. Corkscrew and Jackson orchards are older, smaller and located next to large undeveloped tracts of land. These conditions provide natural vegetation that primary or alternative hosts use. Finally, cultural practices may have influenced mortality types. Grove managers at Silver Strand orchard used Logic® for ant control in 1996. Baiting may have removed the generalist *S. invicta*, allowing indigenous predatory ants to fill the void. This might account for increased mortality due to ants at Silver Strand orchard.

In summary, predation was the most important mortality factor among natural enemies. Greatest defined predation resulted from foraging Formicidae, probably *P. gracilis* and *C. ashmeadi*. *A. citricola* is the most important mortality factor among parasitoids. Parasitism was heaviest from *A. citricola* in the fall of both years. The most

abundant indigenous parasitoid collected was *P. minio*. Indigenous parasitoids were more abundant in spring, declining to low but detectable levels the remainder of the year.

Recovery of leafminers and mortality types indicate that orchard architecture (site) may be an important factor in *P. citrella* survivorship. Therefore, a study was designed to examine orchard architecture and how it affects leafminer survival. The results of this study are presented in the following chapter.

Table 3-1. Time of year, number of cohorts, and total number of marked *P. citrella* eggs placed in groves in 1994, 1996 and 1997.

Year	Date	# Cohorts/Trt ¹	# <i>P. citrella</i>
1994	11 Aug - 23 Aug	1A	37
	26 Aug - 15 Sep	1A	54
	7 Oct - 21 Oct	2A	314
1996	27 Feb - 31 Mar	1A	106
	21 Mar - 4 May	2A, 2AF	423
	14 May - 1 Jun	1A, 2AF	325
	6 Jun - 1 Jul	2A, 2AF	417
	4 Jul - 18 Jul	2A, 2AF	417
	29 Jul - 11 Aug	2A, 2AF, 1T	342
	10 Oct - 26 Oct	3A, 3AF	631
	18 Nov - 4 Dec	2A, 2AF	412
1997	26 Mar - 10 Apr	2A, 2AF, 1T	730
	5 May - 15 May	3A, 3AF	711
	18 Jun - 1 Jul	3A, 3AF	613
	10 Jul - 24 Jul	2A, 2AF, 1T	420
	25 Jul - 8 Aug	2A, 3AF	522
	29 Sep - 12 Oct	3A, 3AF	263

1. A = Ant; AF = Ant Free; T = Established Tree.

Table 3-2. Percent mortality of *P. citrella* by each factor from cohorts in 1994, 1996, 1997 from the Silver Strand, Corkscrew, Everglades and Jackson orchards. Total number of observed individuals = 6,741; 405 in 1994, 3,190 in 1996 and 3,146 in 1997.

Mortality factor	1994	1996	1997	Combined
Ant Predation	39.5	24.9	31.6	29.7
"Heat Stress"	24.1	16.7	15.2	17.4
<i>A. citricola</i>	10.1	15.3	17.3	15.9
Undetermined Predation	-	9.2	7.1	7.6
Indigenous Parasitoids	3.9	4.9	2.1	3.8
Host Feeding	-	2.5	1.5	1.9
Aranea	-	2.2	0.9	1.5
Interference	5.6	0.7	1.5	1.5
Additional	-	1.8	1.1	1.3

Table 3-3. Parasitism on *P. citrella* cohorts on potted trees by species in 1994, 1996 and 1997 from Silver Strand, Everglades, Corkscrew and Jackson orchards.

Species	1994	1996	1997	Total	%
Parasitism	44	439	512	995	100
<i>Ageniaspis citricola</i>	34	333	444	811	81.5
<i>Pnigalio minio</i>	6	18	12	36	3.6
<i>Elasmus tischeriae</i>	0	8	8	16	1.6
<i>Horismenus</i> spp.	3	17	3	23	2.3
Undetermined species	1	63	45	109	10.9

Table 3-4. Type of survivorship curves of *P. citrella* by month along with the r-squared value of its regression by time for each cohort in 1994, 1996 and 1997 from Silver Strand, Everglades and Corkscrew orchards.

Year	Month	Type ¹	R-squared ²
1994	Aug	IV	0.89
	Sep	III	0.91
	Oct	III, III	0.96, 0.93
1996	Mar	III	0.95
	Apr	II, II, II, II	0.95, 0.87, 0.94, 0.96
	May	II, II, II	0.96, 0.94, 0.93
	Jun	IV, III, II, II	0.95, 0.96, 0.98, 0.89
	Jul	III, II, IV, II	0.94, 0.91, 0.93, 0.89
	Aug	II, II, II, II, II	0.92, 0.95, 0.91, 0.84, 0.92
	Oct	I, I, I, I, I, I	0.92, 0.92, 0.84, 0.93, 0.94, 0.92
	Nov	I, I, I, I	0.84, 0.96, 0.96, 0.89
1997	Apr	II, II, II, II, II, II, II	0.92, 0.92, 0.87, 0.87, 0.89, 0.95, 0.95
	May	III, III, II, II, II, III, II	0.88, 0.85, 0.96, 0.91, 0.93, 0.96, 0.93
	Jun	II, II, III, II, II, III	0.94, 0.92, 0.92, 0.83, 0.96, 0.81
	Jul	IV, III, II, II	0.97, 0.97, 0.89, 0.88
	Aug	II, II, II, II, II	0.90, 0.94, 0.95, 0.91, 0.96
	Oct	I, II, I, I, I	0.93, 0.88, 0.95, 0.91, 0.88, 0.89

- Survivorship curve types follow the listing within each month with the mortality tables in the appendix.
- P-Value < 0.001.

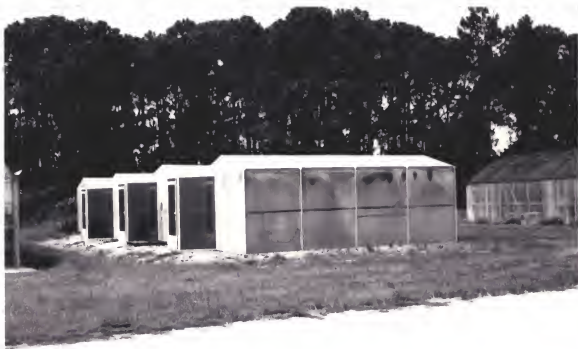


Figure 3-1. Mesh screen house used to allow potted citrus trees to flush free from *P. citrella*.



Figure 3-2. Cages used to infest newly emerging flush with *P. citrella*.

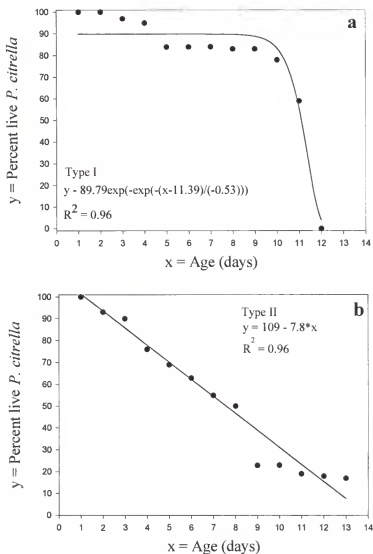


Figure 3-3. Examples showing each of the four regression equations used to determine the type of survivorship curve for each *P. citrella* cohort in 1994, 1996 and 1997. Equations for each curve with their actual constants are shown on each figure along with the type of curve produced. (a) Type I curve data from October 1996 cohort; (b) Type II curve data from April 1996 cohort; (c) Type III curve data from June 1996 cohort; (d) Type IV curve data from June 1996 cohort.

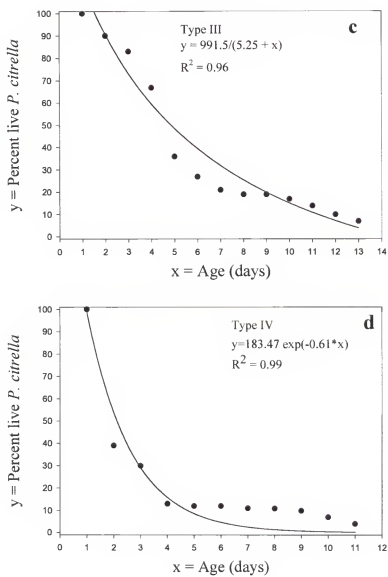


Figure 3-3--continued.

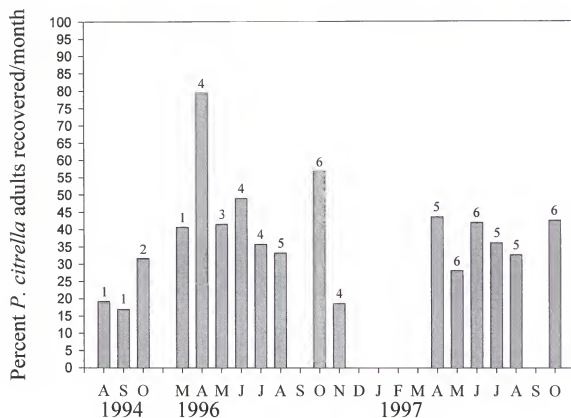


Figure 3-4. Percent of *P. citrella* surviving to adult from cohorts in 1994, 1996 and 1997 at all locations. Total number of individuals observed each month is shown in table 3-1. Number of cohorts observed monthly is shown above each bar. No cohorts were monitored in September and December, 1996 and January, February, March and September, 1997.

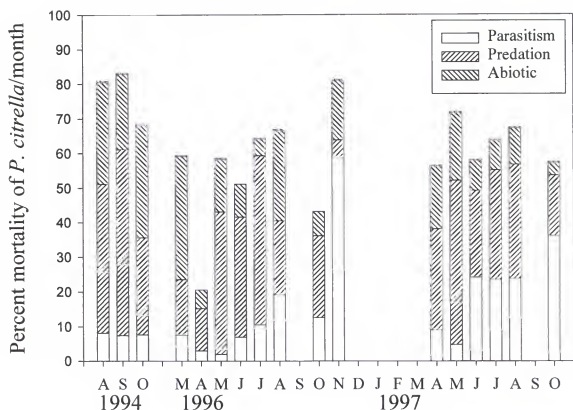


Figure 3-5. Percent *P. citrella* from cohorts killed by mortality category in 1994, 1996 and 1997. Predation is the combined mortality from ants, spiders and undefined predation. Parasitism is the combined mortality of *A. citricola*, indigenous parasitoids and host feeding. Abiotic mortality is wind and "heat stress" combined. Cohorts and locations combined for each month. No cohorts were monitored in September and December, 1996 and January, February, March and September, 1997. Total number of individuals killed in 1994: August n = 30, September n = 45, October n = 262; 1996: March n = 73, April n = 47, May n = 132, June n = 221, July n = 268, August n = 303, October n = 276, November n = 341; 1997, April n = 424, May n = 512, June n = 357, July n = 274, August n = 283, October n = 154. Total number of individuals observed each month is shown in table 3-1.

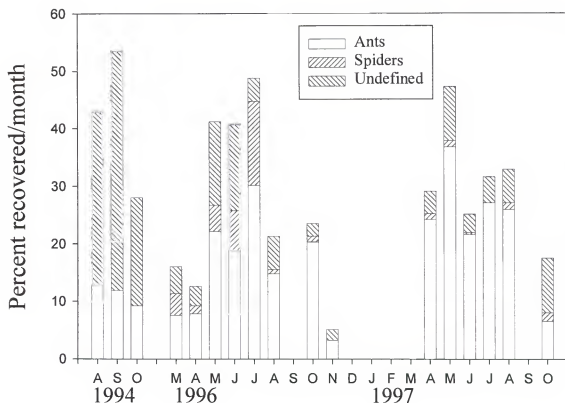


Figure 3-6. Contribution to *P. citrella* mortality from predators (shown in Figure 3-4) indicating percentage killed by ants, spiders and undetermined mortality from cohorts in 1994, 1996 and 1997. Cohorts and locations combined for each month. No cohorts were monitored in September and December, 1996 and January, February, March and September, 1997. Total number of *P. citrella* killed/month by the predator guild in 1994: August n = 16, September n = 29, October n = 88; 1996: March n = 17, April n = 25, May n = 134, June n = 149, July n = 133, August n = 95, October n = 148, November n = 21; 1997, April n = 213, May n = 337, June n = 154, July n = 133, August n = 135, October n = 46.

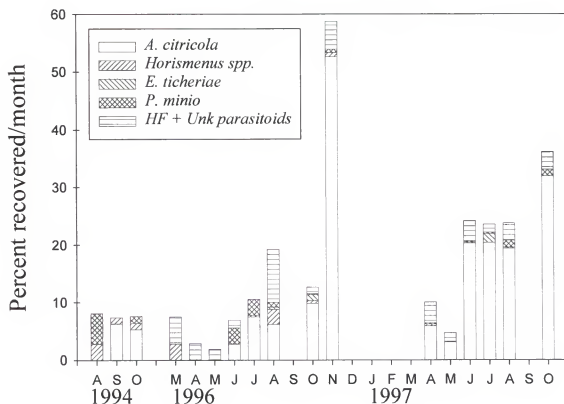


Figure 3-7. Contribution to *P. citrella* mortality from parasitoids (shown in Figure 3-4) indicating percentage killed by *A. citricola*, *Horismenus* spp., *E. ticheriae*, *P. minio* and host feeding + unknown parasitoids in 1994, 1996 and 1997. Cohorts and locations combined for each month. No cohorts were monitored in September and December, 1996 and January, February, March and September, 1997. Total number killed by month in 1994: August n = 3, September n = 7, October n = 40; 1996: March n = 8, April n = 9, May n = 8, June n = 53, July n = 38, August n = 87, October n = 72, November n = 242; 1997, April n = 66, May n = 33, June n = 150, July n = 104, August n = 97, October n = 95.

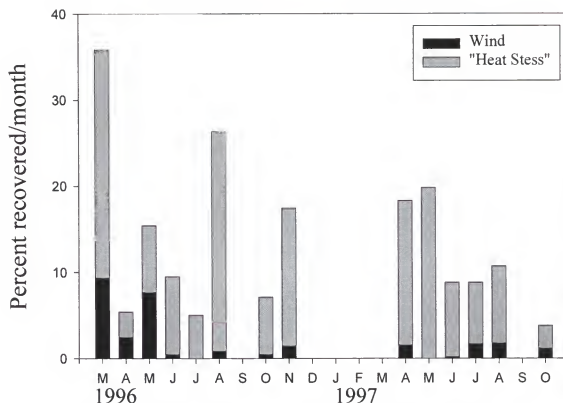


Figure 3-8. Contribution to *P. citrella* mortality from abiotic factors (shown in Figure 3-4) indicating percentage killed by wind and "heat stress" in 1996 and 1997. Cohorts and locations combined for each month. No cohorts were monitored in September and December, 1996 and January, February, March and September, 1997. Total number of *P. citrella* killed/month 1996: March n = 48, April n = 16, May n = 18, June n = 42, July n = 215, August n = 122, October n = 48, November n = 78; 1997, April n = 145, May n = 141, June n = 55, July n = 42, August n = 51, October n = 13.

CHAPTER 4

INCIDENCE OF NATURAL ENEMY COMPONENTS AND THE EFFECT OF GROVE STRUCTURE

Introduction

The wide array of natural enemies of *P. citrella* has been noted previously (Hoy and Nguyen 1997, Browning and Peña 1995, Peña et al. 1996, Ding et al. 1989, Rao and Ramamani 1966, Ujiye and Morakote 1992, Ujiye 1988, Narayanan 1960, Smith et al. 1997). Most authors focus study on a few select species used for classical biological control (Hoy and Nguyen 1997). Several studies have examined indigenous parasitoid species occurring in citrus growing regions recently invaded by *P. citrella* (Peña et al. 1996, Browning and Peña 1995, Perales-Gutiérrez et al. 1996, Bautista-Martinez 1998). For example, Peña et al. (1996) state that by limiting insecticidal sprays, indigenous parasitoids could become a significant mortality factor in south Florida. Predation of citrus leafminer by the green lacewing *Chrysopa boninensis* has been examined in China (Chen et al. 1989). These studies were limited to individual mortality factors of *P. citrella*, or at most predator or parasitoid guilds. No study thus far has examined an entire natural enemy complex attacking *P. citrella*.

Exclusion methods have often been used to assess the impact of natural enemies under field conditions (Kidd and Jervis 1996, Southwood 1978, Van Driesche and

Bellows 1996). Specifically, cage exclusion has been used to isolate and measure the effects of a single component of mortality from an entire natural enemy complex (Luck et al. 1988, Van Driesche and Bellows 1996, Jervis and Kidd 1996). For example, Debach and Huffaker (1971) used open and closed mesh cages to evaluate the effects of parasitoids on California red scale. Neuenschwander et al. (1986) also used open and closed cages to evaluate the effect of *Epidinocarsus lopezi* (De Santis) on the cassava mealybug and O'Neil and Stimac (1988) used small cages to exclude predators of the velvetbean caterpillar, *Anticarsia gemmatilis* Hübner, from soybean.

Various natural enemies attack *P. citrella* in southwest Florida (Chapter 3). Based on previous studies, the best method of evaluating a single species or group of species involves excluding as many mortality factors as possible. By taking advantage of different life history strategies (Chapter 3), the effect of indigenous parasitoids might be isolated from that of *A. citricola*.

If dispersal of *A. citricola* had been less rapid, its effects on *P. citrella* might have been determined by comparing an orchard with *A. citricola* to one without. In lieu of this comparison, larvae could be protected from extraneous forms of mortality to evaluate parasitism by *A. citricola*. This is possible because parasitized *P. citrella* eggs must hatch, develop and form a pupal chamber before *A. citricola* can pupate. By protecting developing *P. citrella* from indigenous parasitoids, a measure of possible interference could be ascertained. To accomplish this, a series of cages similar in design to those used by O'Neil and Stimac (1988) could be used, and unwanted mortality from indigenous parasitoids excluded.

This study also tests the hypothesis that citrus orchards with taller trees and developed canopies provide a more conducive microclimate for certain natural enemies, in particular *A. citricola*. Smith and Hoy (1995) and Edwards and Hoy (1998) have reported that *A. citricola* survival improved with increasing relative humidity, speculating that establishment might be limited to areas of high relative humidity. Nonetheless, the parasitoid has somehow established in Spain, Israel and parts of Morocco (Edwards and Hoy 1998). Edwards and Hoy (1998) speculate that *A. citricola* is limited to humid micro-habitats in these regions. Florida's high humidity should provide *A. citricola* with ample suitable habitat, and indeed this appears to be the case (Chapter 2).

Even with the establishment of *A. citricola*, newly planted orchards suffer greater damage than established ones from *P. citrella* (Knapp et al. 1995). However, increased damage may not be related to differences in microclimate. One must also consider that newly planted orchards produce large amounts of flush. Constant heavy flushing provides continuous new substrate for *P. citrella* populations to increase. It is reasonable to assume that a large population of *P. citrella* would attract more natural enemies. On the other hand, recovery data, number of releases, and parasitism levels from release sites in 1994 to 1996 show that *A. citricola* was difficult to establish in newly planted versus established orchards (Chapter 2). Given the amount of flush, number of times small trees flush, and the abundance of *P. citrella* found on small trees, one would expect *A. citricola* to readily establish and be recovered in newly planted orchards unless other factors were not favorable. Unsuitable microclimate may have resulted in longer establishment times and reduced recoveries of *A. citricola* in newly planted orchards. The objectives of this

study were to examine the interaction between indigenous parasitoids and *A. citricola*, and to ascertain whether orchard architecture and its effect on microclimate influence the major mortality factors of *P. citrella* in southwest Florida.

Materials and Methods

Colonies of citrus leafminer were maintained following the procedures of Smith and Hoy (1995) (Chapter 3, page 24). Preparation of potted citrus trees suitable for oviposition was described (Chapter 2, page 22).

Cages were designed to hold nine potted citrus trees. Cages were made from square aluminum tubing with outside dimensions measuring 5 cm by 5 cm. All tubing had a groove running along the outer edge of one side to receive screening spline. Five panels, four sides and top, were screened with 52 x 52 saran polyethylene screen (0.3 mm openings) (Lumite, 6525 The Corners Parkway, Suite 115, Norcross GA 30092) (Figure 4-1). Outside cage dimensions were 1 m x 60.8 cm x 60.8 cm.

Three orchards of 'Hamlin' oranges, *Citrus sinensis* (L.) Osbeck, were used for this study. One orchard located on the Southwest Florida Research and Education Center (SWFREC), north of Immakolee, Florida in Collier County, was divided into three blocks. The orchard consisted of 326 trees/ha (132 trees/acre), averaging 2 yrs old (planted in 1996) and planted 4.56 x 6.68 m apart, averaging 0.89 ± 0.15 m in height ("short trees") (N=10) (mean $\pm$ SD). Bermudagrass, *Cynodon dactylon* (L.) Pers. was the primary plant cover. Because the same scion was desired for all tree sizes, two different orchards were needed for medium and tall trees. Two medium and two tall height blocks were located at

Silver Strand North in Collier county. The remaining medium and tall height blocks were located at Corkscrew orchard, south of highway 850 in Collier county. Medium height blocks had 372 trees/ha (151 trees/acre), planted 3.64×7.29 m apart with tree heights averaging 3.92 ± 0.33 m ($N = 10$). Tall height groves were planted at the same spacing and averaged 5.25 ± 0.38 m tall ($N = 10$). The tall and medium height blocks at Silver Strand were 15 and 7 yrs old, respectively. The tall and medium height blocks at Corkscrew were 30 and 7 yrs old, respectively. The primary ground cover at Silver Strand was a combination of Bermuda and Bahia grass, *Paspalum notatum* Fluegge, along with native grasses, forbs and grass-like vegetation that had reestablished. Primary ground cover at Corkscrew consists of reestablished native vegetation. All sites had retention ponds and irrigation canals.

Standard grove management practices were conducted at each site during the study. Groves were mowed 3 to 4 times a year. Herbicides were applied approximately 4 times a year. Oil and copper were applied twice a year for control of greasy spot and rust mite (*Phyllocoptruta oleivora* (Ashm.)).

Once a month, from May to November 1997, 36 potted citrus trees were stripped of all leaves, fertilized and watered to stimulate new growth. Trees were then randomly assigned to one of the nine blocks. Plants were watered by tapping into the grove's automatic watering system and placing a micro-sprinkler with an inverted emitter into each pot.

Four pots, placed in groups of two, were exposed to *P. citrella* in each block. When first instar *P. citrella* were found on a set of potted trees in a block, a cage was

placed over one randomly chosen subset of two trees. The remaining subset of two trees was left uncaged. Potted trees were monitored daily until *P. citrella* pupae were observed. Immediately following the discovery of pupating *P. citrella*, potted trees were removed and returned to the laboratory for examination. Three stems from each plant was chosen at random, 18 stems/treatment and 36 stems/block, for a total of 108 stems. All *P. citrella* life stages were examined under a stereo microscope (Leica, Wild M3Z). *P. citrella* were counted and categorized as living or dead. Dead *P. citrella* were assigned to one of three mortality types; predation, parasitism, or "heat stress" based on categories developed for the previous study (Chapter 3, pages 25-26). Parasitism was further divided into *A. citricola* or indigenous parasitoids (Chapter 3, page 25).

Weather data were recorded from within a tall height grove (Corkscrew orchard) and an equivalent of a short height grove (SWFREC). The data logger for the short height grove was located in an adjacent open field. Although these are not the actual conditions of the orchard, it provided an approximation for the conditions that occur in the short height orchard. Climatic data were recorded using Campbell data loggers (Model CR-10, Campbell, Logan, UT) programed to sample humidity (HMP35C probe) every ten seconds at both sites. Humidity was then averaged every hour and the hourly average output.

Chi square analysis was used to examine differences in relative occurrence of the various mortality factors between orchards (PROC FREQ, SAS Institute Inc. 1998). Effects of exclusion cages on mortality factors were analyzed using analysis of variance (ANOVA) with means separated using the least significant difference (LSD) (SAS

Institute Inc. 1988). A t-test was used to determine if any difference in total number of *P. citrella* collected occurred between sites and if any difference in the recovery of mortality factors was different between caged and un-caged potted plants at each site (PROC FREQ, SAS Institute Inc. 1998).

Results

Exclusion Study

Cages effectively excluded indigenous parasitoids. Significantly more *P. citrella* were killed by indigenous parasitoids on uncaged potted plants (Table 4-1). This difference occurred despite the fact that ants were observed foraging on potted plants in both treatments. No other differences between caged and uncaged treatments were observed (Table 4-1).

Location Comparisons

Relative humidity at SWFREC was lower throughout the study compared to Corkscrew orchard (Figure 4-3). Average daily low relative humidity at SWFREC and Corkscrew was 52.21 ± 10.89 and $82.83 \pm 6.67\%$ (mean $\pm$ SD), respectively.

Total number *P. citrella* collected was significantly different between sites with fewer being sampled at Corkscrew orchard compared to Silver Strand and SWFREC orchards ($t = 1.96$; $df = 249$; $P < 0.05$) (Table 4-2). No significant difference was observed in mortality factors between caged and un-caged treatments at individual sites and therefore treatments at each site were combined ($P > 0.05$).

A greater percentage of *P. citrella* adults were recovered at SWFREC and Silver Strand compared to Corkscrew orchard (Chi-square, $P < 0.05$; Table 4-2). Unlike adult emergence, no difference in proportion of live, un-parasitized *P. citrella* larvae was observed between sites (Table 4-2). This may be due to differences between mortality factors occurring at each site and the developmental stage of *P. citrella* they attack.

As had been suspected, a higher percentage of *A. citricola* adults were recovered from tall compared to short height trees. Silver Strand and Corkscrew had more *A. citricola* recovered compared to SWFREC orchard (Table 4-2). In addition, a greater percentage of indigenous parasitoids was recovered at Corkscrew compared to Silver Strand and SWFREC orchards (Table 4-2).

Silver Strand had the lowest percentage of predation compared to SWFREC and Corkscrew. However, no significant difference in predation was observed between Corkscrew and SWFREC orchards over the study period (Table 4-2). The percentage of *P. citrella* killed by "heat stress" was significantly different between SWFREC and Silver Strand orchards (Table 4-2).

Block Height Comparisons

Difference in mortality factors due to block height were compared at Corkscrew and Silver Strand orchards, the only sites with different block heights. When mortality factors were compared at Corkscrew orchard, only predation was significantly different. Predation was higher in medium height (4.57 ± 6.98) (mean $\pm$ SD) versus the tall height block (0.82 ± 1.98) ($t = 2.73$; $df = 54$; $P < 0.05$). Only "heat stress" was significantly different between block heights at Silver Strand orchard. More dead *P. citrella* were

recovered from the tall height blocks at Silver Strand then the medium height block ($t = 2.27$; $df = 110$; $P < 0.05$).

Discussion

Several indigenous parasitoid species have expanded their host range to include *P. citrella* since its introduction into Florida (Schauff and LaSalle 1996). Initial findings showed parasitism levels as high as 50% (Chapter 2, Knapp et al. 1995). Most if not all indigenous parasitoids using *P. citrella* in Florida are generalists. As generalists they likely do not distinguish between previously-parasitized and un-parasitized hosts. This would ultimately lead to the destruction of some *A. citricola*. Knipling (1995) states that parasitism that preempts successful parasitism by another parasite adds no significant contribution to natural control. Therefore, it is important to know just how much interference to *A. citricola* is caused by indigenous parasitoids.

Cages successfully excluded indigenous parasitoids from potted plants. Only 3 indigenous parasitoids were recovered from caged plants, compared to 36 from un-caged plants. Despite this, the mean number of *A. citricola* recovered from caged and uncaged plants was not significantly different, indicating that indigenous parasitoids did not cause interference (Table 4-3). If interference had occurred, fewer *A. citricola* should have been observed on un-caged plants where the greatest numbers of indigenous parasitoids were recovered.

Canopy structure can create a unique microclimate based on tree height (Unwin and Corbet 1991, Cloudsley-Thompson 1962). Short trees with reduced canopy structure

allow greater light and wind penetration, lowering humidity compared to large trees with closed canopy structure, with reduced light penetration and higher humidity (Cloudsley-Thompson 1962).

Knapp et al. (1995) and Stansly et al. (1996) observed that nursery stock and resets were at the greatest risk of *P. citrella* attack. These authors state that increased attack is the result of constant flushing, providing an unlimited food supply for *P. citrella*. It is therefore not surprising that *P. citrella* was recovered in greatest numbers at SWFREC. In addition, *P. citrella* did not appear to be impeded by lower humidity, with the number of leafminer and percentage survivorship being highest at SWFREC (Table 4-1).

A. citricola appeared to be affected by the microclimate created by orchard height. Lowest recoveries, proportionately, of *A. citricola* occurred at SWFREC, while highest recoveries were observed at Corkscrew orchard. With large numbers of *P. citrella* at SWFREC being observed, it seems unlikely that *A. citricola* was limited by host availability. Rather it appears that reduced canopy height at SWFREC created an inhospitable microclimate. Daily low humidity at Corkscrew averaged 82% during the study compared to 52% at SWFREC (Figure 4-2). Edwards and Hoy (1998) showed that under laboratory conditions survival of *A. citricola* adults increased with increasing humidity. *A. citricola* release data also support the importance of humidity. *A. citricola*, released as adults, established quickly in older groves with closed canopies and higher humidity, compared to young groves with little canopy cover (Chapter 2).

Recovery of indigenous parasitoids was influenced by orchard location with a greater percentage of indigenous parasitoids being recovered at Corkscrew compared to SWFREC and Silver Strand orchards (Table 4-2). These findings may be due to differences in surrounding vegetation at Corkscrew orchard. Host ranges are poorly known for indigenous parasitoids but may include leafminers in two or more insect orders (Chapter 3, page 40-41), including leafminers found in surrounding vegetation. Corkscrew is an older orchard with a more diverse ground cover and is adjacent to a large tract of native vegetation (Corkscrew Swamp Sanctuary in Collier Co.). Two plants typically found in and around groves are burnweed, *Erechtites hieracifolia* (L.) Raf., and wild grape, *Vitis rufoomentosa* Small (personal observation). These two plants harbor potential hosts of indigenous parasitoids, *Phyllocnistis vitifoliella* (Chambers) from wild grape and *Phyllocnistis erechtiisella* Chambers from burnweed (Kimball 1965). Many examples exist demonstrating the importance of adjacent vegetation in cropping systems (Zangger et al. 1994, Boller et al. 1988, Smith and Papacek 1991, Douth and Nakata 1973, Stary 1983 and Zhang and Olkowski 1989). Row middles and retention areas provide harborage for indigenous natural enemies and their hosts. As more diverse ground cover develops in and around an orchard similar to Corkscrew, the indigenous parasitoid fauna could become more abundant and diversified. However, it does not appear that this would greatly increase mortality of *P. citrella*, given the low percentage of mortality attributed to indigenous parasitoids.

Tree height and site did influence predation (Table 4-2). Corkscrew not only had the highest percentage of predation compared to the other sites, more predation occurred

in the medium compared to the tall height trees. Nesting sites and food availability may explain why Corkscrew was different from the other sites. The tall height block at Corkscrew had extensive resets and dead trees. Abundant dead trees with hollowed out trunks and limbs may create more nesting sites, allowing ant colonies to occur in closer proximity to *P. citrella*. Two ant species previously identified feeding on *P. citrella* are tree nesters, *P. gracilis* and *C. ashmeadi*, that develop colonies in dead trees and tree limbs (Deyrup and Trager 1986, and personal observation) (Chapter 3). In addition, greater predation may have taken place at Corkscrew because of an abundant prey base. Although medium height trees do not flush as often as small trees, they tend to flush more often than tall trees. Thus, the younger the tree the more constant the food base for *P. citrella* and other citrus pests, like aphids.

SWFREC orchard had almost twice the rate of "heat stress" as the other orchards (Table 4-2). Canopy structure at SWFREC allowed potted plants to be directly exposed to the sun, while increased canopy structure of medium and tall height groves shaded potted plants. This suggests that direct exposure to the sun may be an important mortality factor of *P. citrella*. However, when block height was compared at Silver Strand, it was found that more *P. citrella* had died in tall height trees (14 individuals) than medium height (5 individuals) trees. All of the *P. citrella* larvae died in late developmental stages. The reason more individuals died in the tall height trees compared to the medium height trees is unknown, but is probably not a direct result of "heat stress". Rather, unrecognized host feeding by indigenous parasitoids may account for the greater number of leafminers killed in the tall height trees.

In conclusion, microclimates in newly planted orchards appeared to lower *A. citricola* recovery compared to medium and tall height orchards. Microclimate and tree height did not appear to affect the presence of indigenous parasitoids. Prey bases related to flushing patterns may influence when and where predators occur. Greater mortality occurred from "heat stress" in short height trees compared to medium and tall height trees.

Table 4-1. Mean ($\pm$ SEM) number and percent *P. citrella* surviving to the adult and larvae stage or dying from different causes in caged and uncaged treatments from May to November 1997 at Silver Strand, Corkscrew and SWFREC orchards (df = 1, 228).

	Caged	%	Uncaged	%	F	P > F
<i>P. citrella</i> (Adults)	1.30 $\pm$ 0.12a	30.4	1.13 $\pm$ 0.13a	28.8	2.96	0.0866
<i>P. citrella</i> (Larvae)	1.59 $\pm$ 0.12a	35.5	1.69 $\pm$ 0.14a	36.7	2.86	0.0920
<i>A. citricola</i>	0.49 $\pm$ 0.09a	12.2	0.46 $\pm$ 0.09a	8.2	0.07	0.7870
Indigenous parasitoids	0.01 $\pm$ 0.03a	<0.01	0.13 $\pm$ 0.01b	0.7	10.28	0.0015
Predators	1.38 $\pm$ 0.10a	20.7	1.21 $\pm$ 0.12a	24.4	1.70	0.1931
"Heat stress"	0.22 $\pm$ 0.03a	1.2	0.19 $\pm$ 0.04a	1.2	0.33	0.5665
Total mortality	1.54 $\pm$ 1.55a	69.6	1.51 $\pm$ 1.40a	71.2	1.94	0.1649

Means within a row followed by the same letter are not significantly different (Gabriel LSD, $P < 0.05$).

Table 4-2. Number of *P. citrella* sampled, percent survivorship (adults and larvae) and percent of each mortality factor observed by orchard from May to November 1997. Treatments at each site combined.

	SW ¹	SS	CS
Total sampled	3793	4358	576 ²
<i>P. citrella</i> (Adults)	32.4	28.7	16.4* ³
<i>P. citrella</i> (Larvae)	37.8	34.2	39.5
<i>A. citricola</i>	3.1*	15.1	15.8
Indigenous parasitoids	0.5	0.3	1.4*
Predation	24.6	20.8*	26.0
"Heat stress"	1.6 ^{SS-SW}	0.9	0.9

Chi-square analysis performed on raw data.

¹SW = South West Florida Research and Education Center; CS = Corkscrew orchard; SS = Silver Strand orchard.

²Corkscrew orchard significantly different from SWFREC and Silver Strand orchards (LSD, $P < 0.05$)

³An "*" indicates orchard was significantly different from the other orchards (Chi-square, $P < 0.05$). For "heat stress" SS-SW indicate that only the proportion of dessication from those two orchards were significantly different (Chi-square, $P < 0.05$).



Figure 4-1. Cages used to excluded indigenous parasitoids from potted plants.

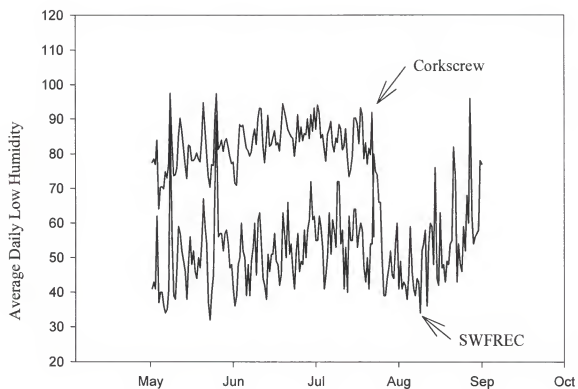


Figure 4-3. Daily low humidity at Corkscrew orchard and Southwest Florida Research and Education Center, Immokalee, Florida from May to November, 1997.

CHAPTER 5 CONCLUDING REMARKS

Summary

Successful establishment and overwintering of *A. citricola* was documented in southwest Florida by 1996. *A. citricola* was chosen for release based on climate matching and successful releases in Australia (Hoy and Nguyen 1997). Establishment of *A. citricola* is a success story for biological control in Florida.

Following establishment of *A. citricola*, mortality factors of *P. citrella* were documented. During preliminary cohort studies in 1994, several indigenous parasitoids (Peña et al. 1996) and predators were found to have expanded their prey base to include *P. citrella*. To evaluate relative effects of different natural enemies, cohorts of *P. citrella* were monitored on potted plants in three orchards in 1996 and 1997.

Predation by ants was the most significant form of natural enemy mortality occurring in cohorts. Mortality occurring to first and second instars classified as “heat stress” was the second most important type of mortality in cohorts. *A. citricola* was the most important parasitoid attacking *P. citrella*, having its greatest impact in late summer and early fall. Indigenous parasitoids did not exert enough pressure to produce high levels of *P. citrella* mortality.

Cohort data showed an apparent relationship between mortality types and orchard architecture. Differences in humidity appeared to affect *A. citricola*, with the lowest percentage of *P. citrella* parasitized at SWFREC. This appears to be caused from differences in microclimate, ground cover and grove management. Orchards with tall established trees have higher humidity compared to newly planted orchards. A higher percentage of indigenous parasitoids was recovered at Corkscrew orchard compared to the other orchards. This may be due to differences in alternate host availability caused by increased diversity of native vegetation. Highest percentage of predation occurred at Corkscrew orchard. This appears to be the result of alternate prey density and orchard architecture. Constant flushing supports alternate prey which may initially attract ants to trees. Finally, orchard height affected the number of leafminers killed by desiccation. A significantly higher percentage of *P. citrella* died from desiccation in the short height orchard of SWFREC compared to the other two orchards.

Study Modifications

During establishment of *A. citricola*, the distance that the parasitoid could disperse was underestimated. This resulted in monitored groves being over sampled for dispersal. Monitoring of adjacent groves earlier would have provided more accurate dispersal information. Had this occurred, releases could have been reduced, or halted in certain areas.

The biggest problem encountered while examining mortality factors of *P. citrella*, was the difficulty in keeping ants off “ant free” trees. Even though Tanglefoot was

painted around “ant free” pots, ants still managed to consume a large portion of *P. citrella* larvae. The best way to avoid this would be total isolation of each plant. Tables with legs immersed in water, along with Tanglefoot painted on pots might stop ants from consuming larvae. The problem with this is that every pot would have to receive the same treatment. This would have been costly and logistically difficult.

Future Studies

With mortality factors of *P. citrella* identified, this study invites further research. More information about indigenous parasitoids is needed. Host range of indigenous parasitoids is not well known. Host range data of indigenous parasitoids could be used in manipulating native vegetation to increase indigenous parasitoids. Recovery data in 1996 and 1997 showed that indigenous parasitoids were more abundant in spring. Increasing surrounding vegetation may augment indigenous parasitoids in spring, lowering *P. citrella* populations earlier in the year.

Conservation of indigenous natural enemies should also be investigated. Data gathered during this study showed predation as the main component of *P. citrella* mortality. Specifically, ants were responsible for most of the predation observed in cohorts. Conservation of these predators can be accomplished in several ways. For example, habitats conducive to ants and their prey could be encouraged. This can be accomplished by reducing the number of times row middles and ditch banks are mowed. This is not a new idea. Long et al. (1998) and Altieri and Whitcomb (1979) have discussed the importance of alternative vegetation to the conservation of natural enemies.

No alternate hosts of *A. citricola* have been documented in Florida. However, on two occasions evidence of possible parasitism by *A. citricola* on two indigenous *Phyllocnistis* spp. was noted. Seven leaves with pupal chambers of *Phyllocnistis meliacella* Becker from a single mahogany tree, *Swietenia mahagoni* (L.) Jacq., were collected on 5 September 1995. The pupal chambers were opened and the contents of each determined. All seven pupal chambers contained pupae resembling that of *A. citricola*. Seventeen pupae were collected, of which 12 had emerged prior to collection. The remaining 5 did not emerge. In addition, three leaves of wild grape, *Vitis rufoomentosa* Small, with one egg of grape leafminer, *Phyllocnistis vitifoliella* (Chambers) on each leaf, were collected in July 1995. Each leaf was individually placed in a petri dish and exposed to a single gravid *A. citricola* female in a no-choice test. In all three tests, *A. citricola* was recorded finding and apparently ovipositing in the grape leafminer egg. Eggs were not followed and it remains unknown if *A. citricola* did oviposit in, or can complete development on this host. Additional research is needed to determine if *A. citricola* can successfully parasitize indigenous *Phyllocnistis* species.

No attempt was made during the study to determine whether *A. citricola* by itself, or in conjunction with other natural enemies, could regulate *P. citrella* below an economic threshold (ET). An ET of 0.74 larva/tender leaf has been proposed by Huang et al. (1989). Peña and Schaffer (1997) developed a sampling plan for *P. citrella* attacking limes in Florida. Using the ET value of Huang et al. (1989) and the sampling method of Peña and Schaffer (1997), a monitoring program needs to be initiated to determine whether *P. citrella* is regulated by its natural enemies. Based on Peña and Schaffer (1997)

the number of developing *P. citrella* should be examined by randomly sampling five flush from five trees for eggs and first instar leafminer during the growing season. By examining the number of eggs and first instars on flush it can be determined whether natural enemies are regulating *P. citrella* below the proposed ET. In addition, monitoring would determine whether *P. citrella* is being regulated throughout the year. If it is decided that at certain times of the year *P. citrella* is not being regulated by natural enemies, steps could be initiated to assist natural enemies. These may include, but are not limited to, increasing alternate hosts of *A. citricola* and indigenous parasitoids, altering cultural practices, and modifying pesticide programs.

APPENDIX
LIFE EXPECTANCY TABLES FOR OBSERVED COHORTS IN 1994, 1996 AND
1997

Time (x) is number of days each cohort was followed, from the time they were placed into orchards until individuals died or became adults. Number surviving (l_x) was determined by adding all individuals alive on day x . Number dying (d_x) during age x can be calculated by $l_x - l_{x+1}$. Number of individuals alive between age x and $x + 1$ can be found by:

$$L_x = \int_x^{x+1} l_x dx$$

Total number of animal age x units beyond age x , is given by:

$$T_x = L_x + L_{x+1} + L_{x+2} \dots L_w$$

Expectation of life is calculated by:

$$e_x = \frac{T_x}{l_x}$$

Survivorship curves were developed using the information from the l_x column (shown) of each life expectancy table.

Abbreviations at the top of each column correspond to the following:

SS - Silver Strand Orchard
 BC - Berry Corkscrew Orchard
 WJ - Wyan Jackson Orchard
 EH - Everglades Hauling Orchard

AF - Ant Free, ants excluded from pots using Tanglefoot
 A - Ant, ants encouraged to forage on plants

1994	SS, A	BC, A	BC, A	EH, A
Day	August	September	October	October
1	37.00	54.00	107.00	207.00
2	34.00	53.00	100.00	182.00
3	27.00	45.00	89.00	151.00
4	26.00	43.00	75.00	132.00
5	16.00	42.00	46.00	125.00
6	12.00	33.00	30.00	105.00
7	12.00	28.00	26.00	72.00
8	8.00	18.00	18.00	62.00
9	8.00	17.00	9.00	58.00
10	8.00	16.00	9.00	58.00
11	8.00	15.00	7.00	55.00
12	8.00	14.00	7.00	55.00
13	7.00	14.00		55.00
14		14.00		48.00
15		13.00		
16		12.00		
17		1.00		

1996	SS, A	SS, AF	SS, A	SS, AF	SS, A	SS, AF	SS, A
Day	March	April	April	May	May	June	June
1	106.00	101.00	102.00	103.00	117.00	105.00	110.00
2	102.00	86.00	97.00	101.00	117.00	102.00	110.00
3	99.00	66.00	95.00	94.00	116.00	101.00	104.00
4	98.00	83.00	91.00	91.00	87.00	99.00	103.00
5	93.00	82.00	89.00	77.00	102.00	99.00	102.00
6	82.00	81.00	83.00	70.00	92.00	93.00	102.00
7	73.00	59.00	86.00	64.00	80.00	83.00	86.00
8	70.00	71.00	74.00	56.00	69.00	70.00	77.00
9	64.00	68.00	72.00	51.00	48.00	61.00	59.00
10	49.00	66.00	71.00	24.00	47.00	60.00	60.00
11	41.00	63.00	72.00	24.00	42.00	54.00	60.00
12	36.00	58.00	68.00	20.00	40.00	52.00	50.00
13	37.00	54.00	69.00	19.00	39.00	49.00	48.00
14	35.00	53.00	68.00	18.00		47.00	44.00
15	32.00	52.00	68.00			40.00	38.00
16	30.00	53.00	67.00			27.00	16.00
17	30.00	53.00	66.00				
18	28.00	52.00	86.00				
19	26.00	50.00	49.00				
20	26.00	48.00	58.00				
21	26.00	40.00	58.00				
22	25.00						
23	24.00						
24	24.00						
25	25.00						
26	20.00						
27	20.00						
28	15.00						
29	12.00						
30	4.00						

1996	BC, AF	BC, A	SS, AF	SS, A	BC, AF	BC, A	WJ, AF
Day	June	June	July	July	July	July	July
1	110.00	110.00	106.00	103.00	110.00	110.00	103.00
2	110.00	107.00	95.00	88.00	105.00	102.00	22.00
3	108.00	105.00	88.00	84.00	105.00	96.00	11.00
4	106.00	101.00	71.00	70.00	90.00	87.00	2.00
5	103.00	96.00	38.00	65.00	77.00	71.00	0.00
6	92.00	90.00	29.00	64.00	75.00	62.00	0.00
7	82.00	39.00	22.00	49.00	62.00	62.00	0.00
8	80.00	68.00	20.00	54.00	48.00	58.00	0.00
9	74.00	67.00	20.00	54.00	45.00	56.00	2.00
10	74.00	63.00	18.00	54.00	33.00	56.00	0.00
11	77.00	63.00	15.00	77.00	87.00	51.00	0.00
12	75.00	14.00	11.00	59.00	78.00	13.00	
13	70.00	14.00	7.00	36.00	18.00		
14	61.00	6.00					
15	40.00						
16	12.00						

	WJ, A	SS, AF	SS, A	BC, AF	BC, A	WJ, AF	WJ, A
Day	July	July/ August	July/ August	July/ August	July/ August	July/ August	July/ August
1	105.00	110.00	93.00	110.00	107.00	110.00	104.00
2	41.00	102.00	86.00	102.00	105.00	98.00	98.00
3	31.00	88.00	84.00	55.00	40.00	62.00	88.00
4	14.00	66.00	70.00	11.00	15.00	35.00	69.00
5	13.00	61.00	47.00	6.00	15.00	32.00	59.00
6	13.00	56.00	48.00	3.00	13.00	30.00	61.00
7	12.00	55.00	44.00	3.00	13.00	28.00	57.00
8	12.00	52.00	43.00	3.00	15.00	25.00	52.00
9	10.00		42.00	2.00	11.00		51.00
10	7.00		24.00				17.00
11	4.00						

1996	BC, AF	BC, A	WJ, AF	WJ, A	BC, T	SS, AF	SS, A
Day	August	August	August	August	August	October	October
1	105.00	100.00	85.00	106.00	51.00	105.00	105.00
2	95.00	92.00	78.00	94.00	51.00	105.00	105.00
3	95.00	86.00	77.00	96.00	50.00	105.00	105.00
4	84.00	62.00	63.00	76.00	43.00	100.00	101.00
5	77.00	61.00	66.00	71.00	29.00	97.00	98.00
6	68.00	47.00	52.00	67.00	26.00	95.00	95.00
7	27.00	21.00	28.00	62.00	21.00	94.00	81.00
8	24.00	20.00	27.00	61.00	16.00	72.00	63.00
9	24.00	17.00	27.00	59.00	16.00	64.00	58.00
10	22.00	14.00	27.00	58.00	14.00	63.00	58.00
11	13.00	3.00	27.00	52.00	13.00	62.00	58.00
12			8.00	11.00	12.00	62.00	56.00
13					2.00	51.00	48.00

	BC, AF	BC, A	WJ, AF	WJ, A	SS, AF	SS, A	WJ, AF
Day	October	October	October	October	Nov	Nov	Nov
1	105.00	100.00	106.00	110.00	100.00	100.00	105.00
2	105.00	100.00	106.00	110.00	100.00	100.00	105.00
3	105.00	100.00	106.00	110.00	97.00	99.00	105.00
4	100.00	89.00	100.00	104.00	95.00	98.00	105.00
5	100.00	85.00	98.00	99.00	84.00	94.00	105.00
6	96.00	81.00	90.00	99.00	84.00	94.00	100.00
7	93.00	75.00	85.00	96.00	84.00	92.00	94.00
8	88.00	57.00	68.00	88.00	83.00	88.00	90.00
9	77.00	51.00	66.00	79.00	83.00	86.00	90.00
10	76.00	47.00	62.00	73.00	78.00	84.00	74.00
11	74.00	45.00	62.00	71.00	59.00	66.00	6.00
12	68.00	38.00	61.00	66.00		1.00	
13			40.00	22.00			

1996		WJ, A						
	Day	Nov						
	1	107.00						
	2	107.00						
	3	107.00						
	4	107.00						
	5	95.00						
	6	91.00						
	7	83.00						
	8	78.00						
	9	77.00						
	10	71.00						
	11	24.00						
1997		SS, AF	SS, A	BC, AF	BC, A	BC, T	WJ, AF	WJ, A
	Day	March	March	Mach	March	March	March	March
	1	105.00	105.00	105.00	105.00	100.00	105.00	105.00
	2	105.00	105.00	105.00	105.00	100.00	105.00	105.00
	3	105.00	103.00	103.00	105.00	100.00	104.00	104.00
	4	105.00	103.00	103.00	105.00	100.00	98.00	101.00
	5	94.00	96.00	100.00	100.00	71.00	94.00	93.00
	6	88.00	88.00	96.00	99.00	65.00	80.00	82.00
	7	83.00	82.00	91.00	92.00	34.00	69.00	76.00
	8	78.00	65.00	87.00	90.00	23.00	57.00	65.00
	9	56.00	41.00	78.00	85.00	22.00	45.00	55.00
	10	37.00	27.00	64.00	69.00	21.00	39.00	55.00
	11	31.00	24.00	64.00	66.00	21.00	35.00	42.00
	12	28.00	24.00	45.00	65.00			
	13	22.00	13.00		44.00			

1997	SS, AF	SS, A	BC, AF	BC, A	BC, T	WJ, AF	WJ, A
Day	May	May	May	May	May	May	May
1	100.00	86.00	105.00	105.00	105.00	105.00	105.00
2	99.00	86.00	105.00	105.00	105.00	97.00	104.00
3	91.00	85.00	104.00	100.00	112.00	90.00	100.00
4	87.00	83.00	98.00	92.00	88.00	80.00	97.00
5	64.00	80.00	78.00	80.00	73.00	59.00	81.00
6	25.00	38.00	71.00	77.00	63.00	40.00	54.00
7	19.00	31.00	65.00	70.00	53.00	39.00	54.00
8	13.00	26.00	57.00	46.00	47.00	34.00	34.00
9	9.00	26.00	36.00	17.00	34.00	31.00	30.00
10	6.00	25.00	23.00	6.00	26.00	29.00	26.00
11	4.00	23.00	20.00	4.00	26.00	22.00	23.00
12	3.00	23.00	20.00	4.00	26.00	20.00	22.00
13	3.00	22.00	19.00	2.00	24.00	16.00	12.00
14	3.00	10.00	7.00	2.00	18.00		
15	2.00		3.00	2.00	9.00		

	SS, AF	SS, A	BC, AF	BC, A	WJ, AF	WJ, A	SS, AF
Day	June	June	June	June	June	June	July
1	100.00	105.00	96.00	105.00	102.00	105.00	105.00
2	100.00	105.00	96.00	105.00	102.00	105.00	105.00
3	93.00	96.00	77.00	91.00	94.00	95.00	79.00
4	77.00	82.00	53.00	79.00	89.00	91.00	42.00
5	57.00	49.00	26.00	71.00	82.00	89.00	36.00
6	53.00	39.00	18.00	63.00	75.00	86.00	30.00
7	48.00	39.00	15.00	60.00	73.00	83.00	28.00
8	40.00	35.00	13.00	59.00	70.00	83.00	12.00
9	40.00	33.00	11.00	55.00	66.00	81.00	11.00
10	31.00	21.00	8.00	55.00	63.00	80.00	11.00
11	30.00	20.00			62.00	38.00	2.00
12	19.00	3.00					

1997	SS, A	BC, AF	BC, A	SS, AF	BC, AF	BC, A	WJ, AF
Day	July	July	July	August	August	August	August
1	105.00	105.00	105.00	90.00	105.00	102.00	100.00
2	88.00	105.00	105.00	90.00	105.00	102.00	100.00
3	58.00	96.00	83.00	90.00	105.00	102.00	100.00
4	33.00	79.00	79.00	90.00	82.00	85.00	98.00
5	20.00	70.00	71.00	52.00	72.00	81.00	90.00
6	16.00	67.00	69.00	50.00	69.00	77.00	79.00
7	13.00	65.00	67.00	38.00	59.00	65.00	53.00
8	7.00	49.00	58.00	34.00	58.00	59.00	33.00
9	6.00	48.00	54.00	1.00	44.00	54.00	21.00
10	6.00	3.00	17.00	1.00	43.00	51.00	18.00
11				1.00	43.00	51.00	16.00
12				1.00	13.00	25.00	15.00
13				1.00			13.00
	WJ, A	WJ, T	SS, A	SS, AF	BC, A	BC, AF	WJ, A
Day	August	August	October	October	October	October	October
1	102.00	23.00	50.00	16.00	50.00	48.00	49.00
2	102.00	19.00	50.00	16.00	50.00	48.00	49.00
3	102.00	16.00	50.00	15.00	46.00	46.00	39.00
4	92.00	3.00	47.00	15.00	46.00	35.00	37.00
5	84.00	3.00	46.00	15.00	46.00	35.00	35.00
6	81.00		42.00	11.00	38.00	30.00	30.00
7	69.00		42.00	10.00	37.00	24.00	31.00
8	61.00		41.00	10.00	30.00	23.00	30.00
9	39.00		14.00	8.00	9.00	2.00	15.00
10	38.00						
11	37.00						
12	35.00						
13	11.00						
	WJ, AF						
Day	October						
1	50.00						
2	50.00						
3	44.00						
4	44.00						
5	41.00						
6	23.00						
7	24.00						
8	24.00						
9	20.00						

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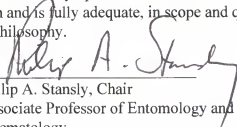
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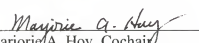
BIOGRAPHICAL SKETCH

Mark Allen Pomerinke was born in Spokane, Washington on July 22, 1968. He grew up in Cheyenne, Wyoming and graduated from Cheyenne East High School in 1986. He received his Bachelor of Science in entomology from the University of Wyoming in 1991 and moved to New Mexico to attend New Mexico State University. In 1994 he received his Master of Science in Agricultural Biology, majoring in entomology and minoring in experimental statistics. Mark married Tracy Tegart in June, 1998.

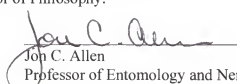
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Philip A. Stansly, Chair
Associate Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Marjorie A. Hoy, Cochair
Eminent Scholar of Entomology and
Nematology

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Jon C. Allen
Professor of Entomology and Nematology

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Crawford S. Holling
Arthur R. Marshall, Jr. professor of Ecological
Sciences

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1999


Dean, College of Agriculture

Dean, Graduate School